Fall | 11

Tissue Fragment Injection System

BME 200/300

Advisor: Dr. Randolph Ashton
J.D. Dorrance, Andrew Osterbauer, Ashley Quinn, and Emma Weinberger

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 hydrosoluable, biodegradable polymer, and uses a biopsy needle to implant them (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. The three proposed designs were weighed in a design matrix based on cost, ease of use, biocompatibility, ergonomics, reliability, and ease of production. Reliability was given the highest priority, closely followed by biocompatibility and ease of use.

The result of this matrix showed that the PLGA capsule would be the best design as it most effectively eliminates unwanted seeding and lowers the technical skill required to perform the procedure. Future work to be done includes testing PLGA encapsulation methods and biopsy needle properties.

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Background:

The liver is the most common site for metastases of gastrointestinal, malignant melanoma, and liver cancer tumors (Lee K-H 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 Shope et al 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 K-H et al, 2009).

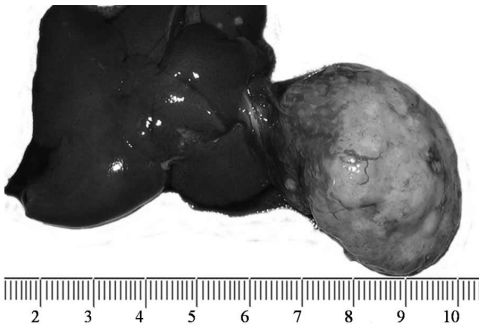

Figure 1: Vx-2 carcinoma tumor 28 days after implantation which displays extensive growth and surface blood vessels (Luo et al, 2010).

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 K-H 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 the rabbits are exposed to. (Luo et al & Lee K-H et al, 2010). Rabbits spend less time under anesthesia and exhibit a recovery time that is 3 times as fast as the surgical method following the percutaneous procedure (Lee K-H 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 K-H et al, unwanted seeding occurred in 21 percent of rabbits when the percutaneous method was used versus eight percent of rabbits when the surgical method was used. 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 biopsy 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, as seen in parts A and B of Figure 2. Next, as pictured in part C of Figure 2, the wire is used to push the tumor cells out of the needle, with guidance by ultra sound imaging for accurate placement. Finally in parts D and E of Figure 2, the 16-gauge needle and wire are retracted into the sheath and the sheath is removed from the animal (Lee K-H 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 (Luo et al, 2010). This needle used in this method is illustrated in Figure 3.

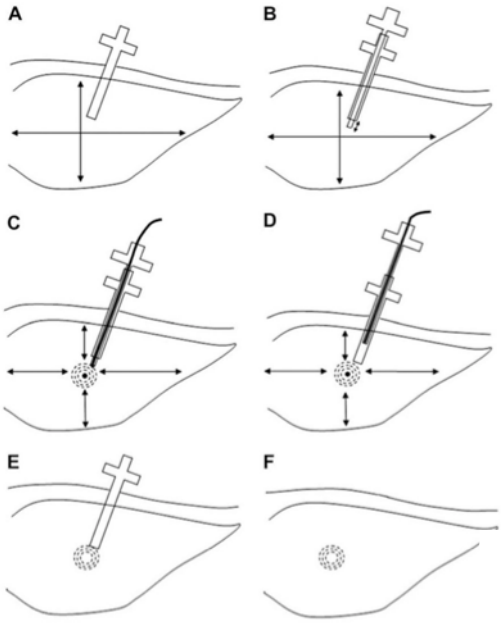

Figure 2: Existing percutaneous method schematic involving a 14-gauge sheath, 16-gauge needle, and wire. Part A: 14-gauge sheath is inserted into rabbit. Part B: 16-gauge needle, loaded with tumor cells, is inserted into sheath. Part C: Wire is inserted into 16-gauge needle and tumor cells are pushed 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).

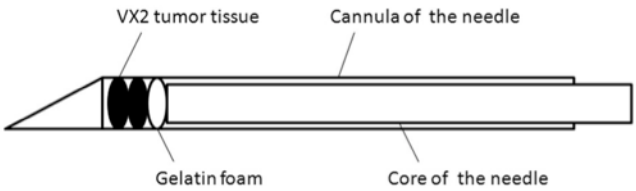

Figure 3: Needle used in percutaneous method which utilizes an 18-gauge needle, sheath, and gelatin foam (Luo et al, 2010).

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 improved 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 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 needle size should not exceed 18-gauge. The device should weigh less than two pounds to allow the user to easily hold the device for the duration of the multiple procedures performed in one day. There is no given maximum budget for the device, but it should be kept within reason; we anticipate the design to cost approximately 85 dollars.

Design Alternatives:

PLGA Capsule:

This procedure would involve encapsulating the tumor fragments in a layer of hydrolyzable 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

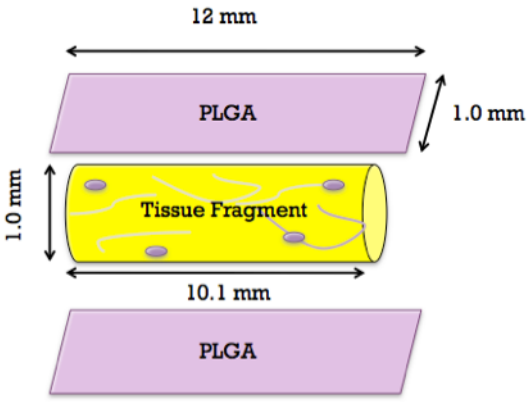

Figure 4. Dimensions and configuration of PLGA capsule. Cells and extracellular matrix can be seen in the tissue fragment.

incompliance, making PLGA a good candidate for our capsule material.

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, 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 dye-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).

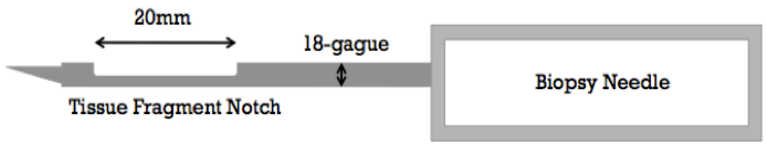

Figure 5: 18-gauge biopsy needle that will be used for injecting the PLGA capsule into the liver. There is a retractable sheath that can be released to cover the 20mm tissue fragment notch.

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.

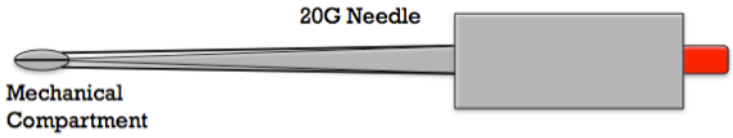

Figure 6. The CDM, image shows the mechanical compartment at the needle tip and the mechanism that that will open and close the compartment.

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 low critical solution temperature (LCST) of 32-37 degrees Celsius, N-IPAAm will under go 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, 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 dye-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 approximately two hours, allowing the cells to interact with the hepatic cellular environment.

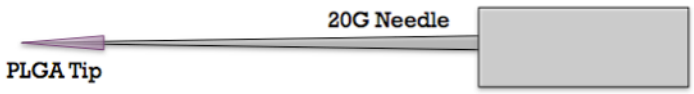

Figure 7. The first 20-gauge needle covered with the PLGA tip.

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 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	6	5	7
Ease of Use	20	15	12	12
Biocompatibility	20	10	8	15
Ergonomics	10	7	7	7
Reliability	30	18	22	6
Ease of Production	10	8	6	4
Total	**100**	**64**	**60**	**51**

Final Design: PLGA Capsule:

The full procedure used for the final design will begin with encapsulating the tissue fragment in PLGA, as described earlier, by dye-casting the PLGA polymer into two sheets and sandwiching the tissue fragment between these two sheets in order to reduce exposure to the insertion pathway. Next, the capsule will be loaded into the tissue fragment notch of the biopsy needle using small forceps. The retractable sheath will then be released to cover the notch, acting as an additional barrier between the cells and abdominal cavity. Using ultrasound visualization, the needle will be inserted into an area in the liver with a thickness greater then 2 cm. Then, the sheath will be retracted, exposing the notch, allowing the capsule to be released from the notch into the hepatic environment. The sheath will then be released over the notch again, sequestering any residual cells remaining in the notch, and the needle will be retracted from the abdomen (Figure 8). Pressure will be applied for 2 to 3 minutes, and the capsule will remain implanted in the liver. Over time as hydrolysis of the PLGA takes place, the tumor cells will be released into the hepatic environment.

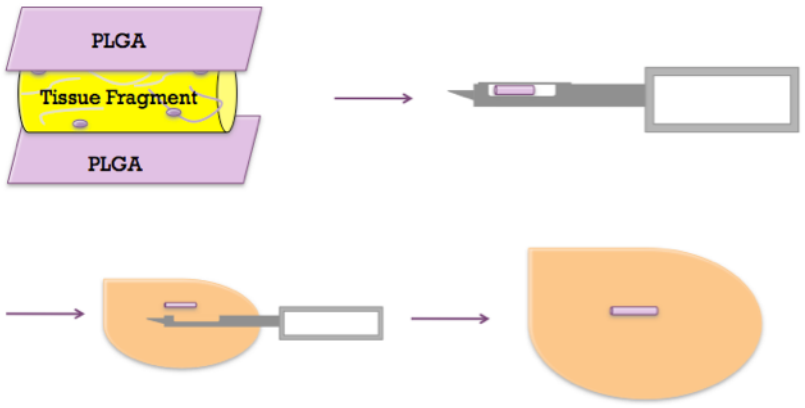

Figure 8: The final procedure is summarized by this flow chart. The tissue fragment is encapsulated with PLGA, loaded into the needle, injected into the liver, and pressure applied following retraction of the needle.

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.

Future Work:

After selection of the PLGA encapsulation design idea, there are several steps to complete in order to create a final working prototype, and prepare this device to be implemented in Dr. Brace's laboratory.

The first step in moving towards a prototype is to order some of the necessary materials. We plan to order PLGA from Sigma-Aldrich and perform some preliminary testing with the material to gain a better understanding of how it interacts with cellular environments and how it will behave in vivo. After extensive research, we have found that PLGA is likely the best material for our purposes; however it is crucial that we experiment with the material at various temperatures, pressures, concentrations, etc. Any observations made about optimal conditions for use of PLGA will allow us to make any necessary adjustments to our design.

Once we have a greater familiarity with the material properties of PLGA, we will need to determine how to best encapsulate the Vx-2 carcinoma cells either by sandwiching the cells between two flat sheets of PLGA or molding the PLGA sheets into a more rounded pellet. We hope to be able to actually perform this dye-casting procedure, however, it is possible that we will not be able to obtain the proper equipment to do so in the remainder of the semester.

Next, we must perform some testing using the biopsy needle that will be used in our design. Before we begin loading the needle with cells, we will practice targeting, injecting, and retracting the needle, using a cow's liver obtained from Dr. Brace's laboratory This will allow us to gain a sense of the speed and force at which the needle will penetrate the liver tissue, allowing for optimization of the protocol.

Finally, each team member will need to obtain Research Animal Resource Center, or RARC, Certification. This certification will allow us to handle the rabbits used for this procedure, and eventually test our design on the rabbits.

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 a biodegradable material 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 biopsy 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

September 12, 2011

Team: Ashley Quinn, Emma Weinberger, James Dorrance, Andrew Osterbauer
Client: Dr. Chris Brace
Advisor: Dr. Randolph Ashton

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 biopsy 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.