Tissue Fragment Injection System

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Background

- The liver is the most common site for metastases of gastrointestinal and malignant melanoma cancer tumors [1]
- Vx-2 carcinoma tumor model is the most common model for studying liver cancer growth and developing potential treatments for humans
  - Fast-growing
  - Similar blood flow
  - Similar genetics

Existing Methods

Open Laparotomy [1,2]
- Most common method
- Easy access to site of implantation
- Accurate placement of cells
- Minimal unwanted cell seeding in abdominal cavity
- Long procedure and recovery time
- Anesthetic complications

Percutaneous [1,3]
- Less invasive
- Shorter procedure and recovery time
- Less anesthesia
- Decrease technical skill required to perform
- Sonographic imaging for guidance
- Increased unwanted seeding of tumor cells
- Difficulty closing internal injection site

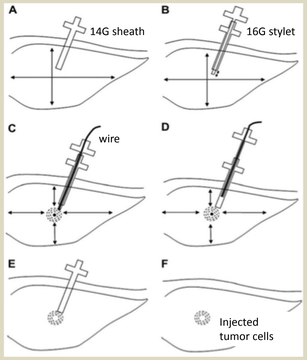


Figure 1: One example of percutaneous injection method (Lee et al., 2009). Includes 14 and 16G needles as well as a wire for ejection of tumor cells.

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.

Materials

Polylactic-co-glycolic Acid (PLGA) [4,5]
- Broken down via hydrolysis mechanisms in the body
- Customizable degradation time
- FDA approved
- Biocompatible
- Durable
- Mechanical flexibility

Poly N-Isopropylacrylamide (pN-IPAAm) [6]
- Thermo-responsive polymer
- FDA approved
- Gels as lower critical solution temperature (32-37°C)

Final Design

Key Design Specifications
- 5mm X 3mm rectangular sheet of PLGA made by electrospinning
- Tissue fragments folded inside of PLGA sheets
  - Prevents unwanted seeding along injection pathway
- 17-gauge coaxial needle for injection
  - Hollow need with blunt stylet that fits inside

Protocol:
- Cut 8mm^3 tissue fragment and 5mm X 3mm PLGA sheet
- Wrap tissue in PLGA sheet
- Backload fragment into needle
- Using blunt tip stylet, push tissue fragment 2cm from tip of sheath
- Stick needle 5cm into liver
- Push stylet through remainder of sheath to eject fragment
- Retract stylet from tip of sheath
- Remove both needles

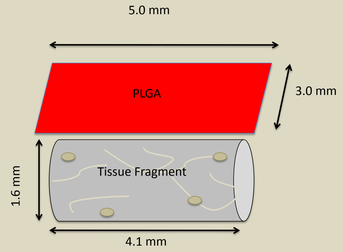
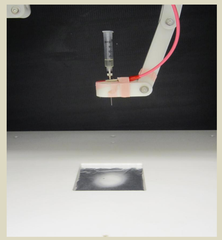


Figure 2. Dimensions and configuration of PLGA capsule. Cells and extracellular matrix can be seen in the tissue fragment.

Testing

Electrospinning Optimization



Figures 3. Electrospinning set-up. Using an 18-gauge needle, 18kV, and a working distance of 16cm, PLGA fibers were spun on to aluminum foil.

Table 1. 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	16cm	poor: little direction
7,000-17,000 kDa	35%	18kV	16cm	N/A (not viscous enough to stay in needle)
7,000-17,000 kDa	50%	18kV	16cm	improved: more direction
100,000 kDa	30%	18kV	16cm	optimal

- Quantified quality of sheet by uniformity of fiber direction, depletion of solvent droplets, linearity of fibers, and amount of overlap between fibers
- Microfiber structure was viewed under a microscope at 20x magnification

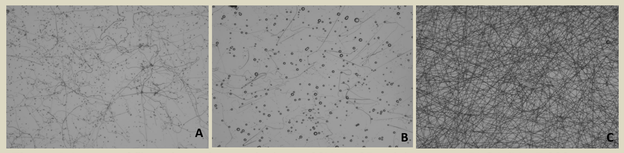


Figure 4. Images of fibers from electrospinning at 20X. **A**. 25 weight percent PLGA (Mw 7,000-17,000). **B**. 50 weight percent PLGA (Mw 7,000-17,000) **C**. 30 weight percent PLGA (Mw 100,000).

Injection Method

Table 2. 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*	*No PLGA*	*PLGA*	*No PLGA*	*PLGA*	*No PLGA*	*PLGA*	*No 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

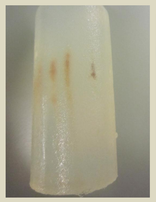


Figure 5. Liver tissue fragments injected into 2% agar phantom. Not encapsulated with PLGA.

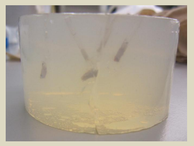


Figure 6. Liver tissue fragments, encapsulated in PLGA, injected into 2% agar phantom.

Design Alternatives

PLGA Capsule
- Encapsulates tumor fragment between two sheets of PLGA
- Delivered via biopsy needle

Cellular Delivery Mechanism (CDM)
- Mechanical device with delivery compartment

PLGA Tip and N-IPAAm Plug
- Needle tip covered with PLGA ejected with tumor cells
- N-IPAAm injected after to fill injection site hole

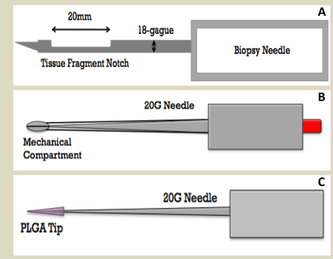


Figure 6. Shown are three different design alternatives. **A**: PLGA Capsule needle design. **B**: Cellular Delivery Mechanism needle design. **C**: PLGA Tip and N-IPAAm Plug needle design.

Cost

Table 3. Estimated cost of 50-100 procedures.

Item	Price
Coaxial Needle	$99.99
1gram PLGA (50:50, Mw 100,000)	$175.00
10mL Tetrahydrofuran (THF)	$4.15
10mL N,N-Dimethylformamide (DMF)	$4.32
TOTAL COST	$283.46

Future Work

- Determine and customize degradation time of PLGA
  - accurate simulation of rabbit liver environment
- Improve efficiency of encapsulation process
  - lower technical skill required
  - decrease time required
- Preliminary research shows promising results for the use of (poly)-N-IPAAm as a plug to injection site
  - continue testing insertion methods and conditions
- Determine optimal way to store sheets of PLGA
  - storage temperature
  - storage time
- Once complete confidence in method and device is achieved, test on recipient rabbits.

References

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