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(New Kim on the Block)

Injectable Polymer Scaffolds – An Approach to Cartilage Tissue Engineering

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Background

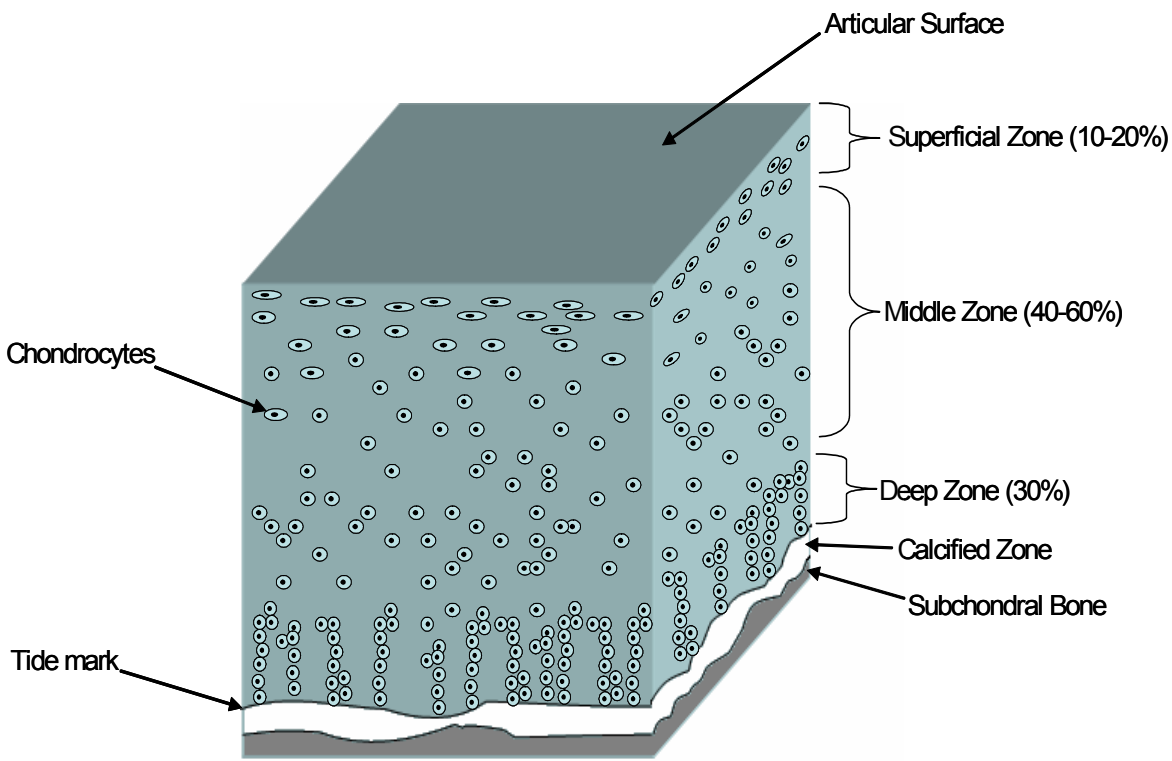
- Millions of Americans suffer from trauma, disease, or malformation of cartilage tissue
- Cartilage provides
  - Mechanical support
  - Distributes forces during loading
  - Lubrication to the joint
- Cartilage lacks the ability to regenerate itself
- Current treatments are not seen with high success rates

Project Aim

- Goal
  - Regenerate cartilage in knee to restore full functionality in a minimally invasive manner
- Pathway
  - Employ a procedure involving an injection of a biodegradable cell/scaffold/growth factor composite into defect site

Cartilage Anatomy

- Composed of four components
  - Chondrocytes
    - 10% of total volume of cartilage tissue
  - Collagen
  - Proteoglycans
  - Water
    - 80% of total volume of cartilage tissue

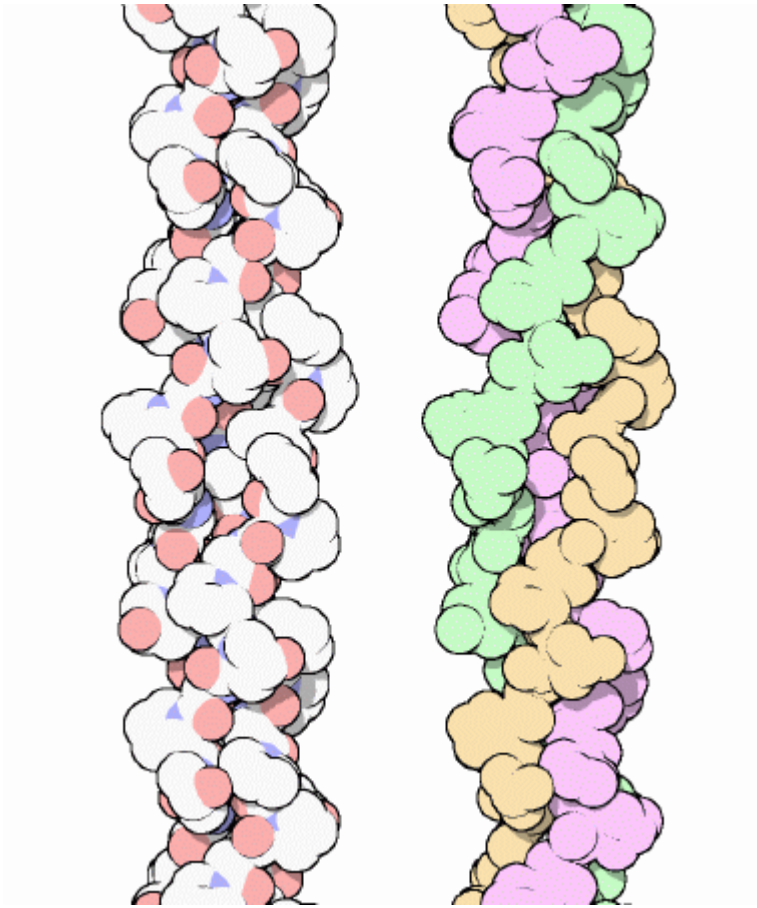


Chondrocytes

- Cartilage receives no neural impulses
  - No nerve supply
  - No vascular supply
- Cannot signal repair

Collagen

- Macromolecule with triple helical structures
- Type II is prevalent in articular cartilage
- Gives cartilage shear and tensile properties
- Maintains proteoglycan in extracellular matrix



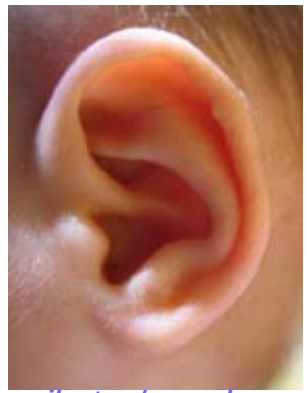
http://www.rcsb.org/pdb/molecules/pdb4_1.html

Proteoglycan

- Complex macromolecules
  - Long protein chain
  - 100s of bound glycosaminoglycans
- Promotes proteoglycan-collagen and proteoglycan-proteoglycan interactions
- Holds the tissue together
- Gives mechanical properties

Types of Cartilage

- Fibrocartilage (type I)
  - Non-load bearing regions
    - Ear
    - Nose
  - Less resilient mechanical properties
- Hyaline cartilage (type II)
  - Prevalent in all diarthroidal joints
  - Resilient mechanical properties

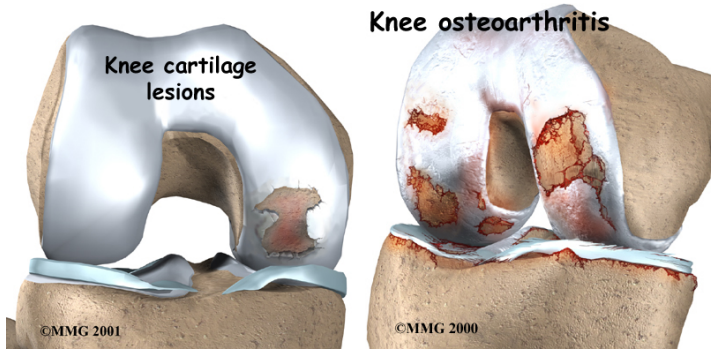


www.silent.se/ soundscapes.php

http://netscape.lhj.com/lhj/story.jhtml?storyid=/templatedata/bhg/story/data/6501.xml&catref=cat410032

Cartilage Defects

- Injury to articular cartilage
  - Cartilage lesions
  - Osteoarthritis



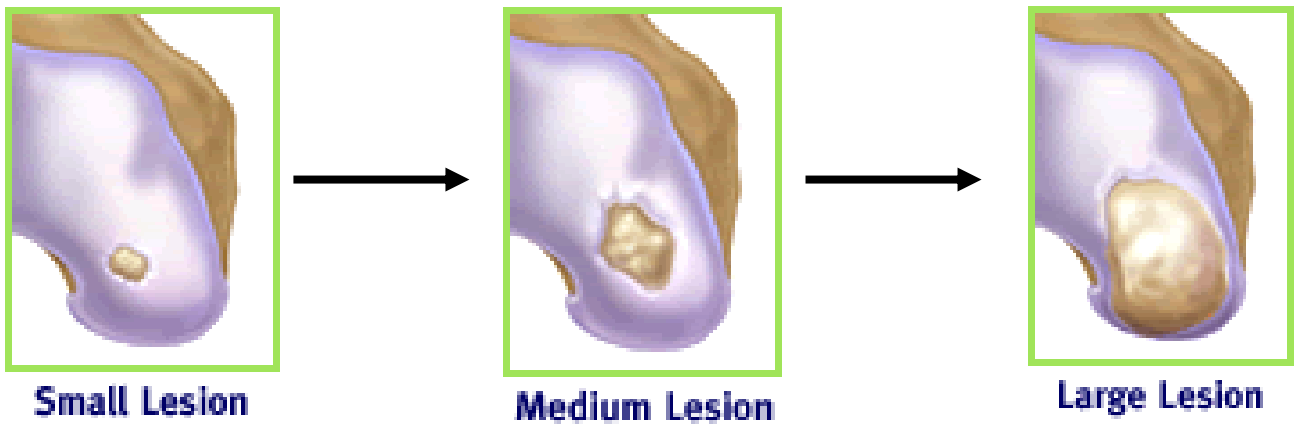
Ref: http://www.orthogastonia.com/patient_ed/html_pages/knee/knee_cartilage_surgery.html

- Cartilage lacks the inherent means to regenerate itself

- Many cartilage defects affect underlying subchondral bone

Cartilage lesions

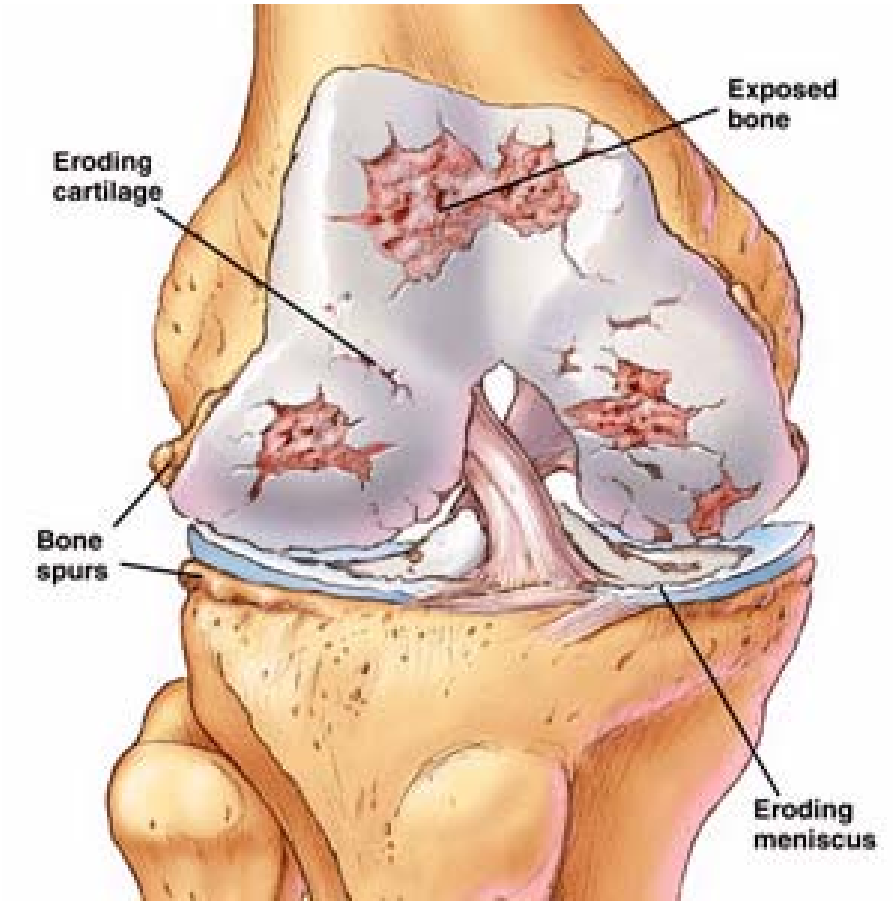
- Underlying bone is often exposed
- May result in unbalanced joint
- Leads to further damage with surrounding tissues



http://www.carticel.com/patients/learning/aboutcartdamage.asp

Osteoarthritis

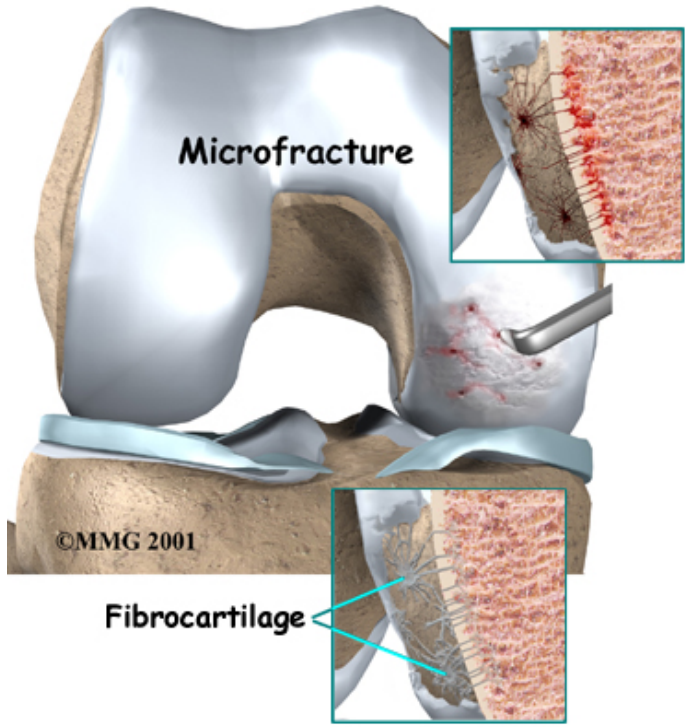
- Noninflammatory degenerative joint disease
- Bones rub and underlying subchondral bone is warn away
- Leads to further complications



http://www.zimmer.com/ctl?op=global&action=1&id=1979&template=PC

Current Therapies

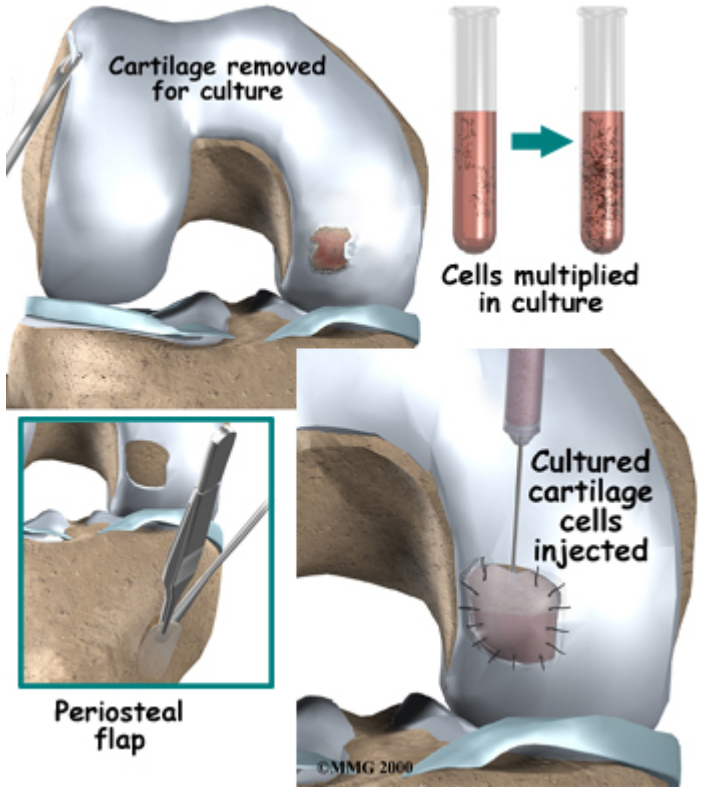
- Reparative
  - Temporary
  - Produces fibrocartilage
    - Arthroscopic debridement
    - Abrasion arthroplasty
    - Microfracturing



http://www.orthogastonia.com/patient_ed/html_pages/knee/knee_cartilage_surgery.html

Current Therapy

- Restorative
  - Not highly successful
  - Chondrocytes migrate from defect site
    - Osteochondral autografts
    - Osteochondral allografts
    - Autologous chondrocyte implantation



http://www.orthogastonia.com/patient_ed/html_pages/knee/knee_cartilage_surgery.html

Solution

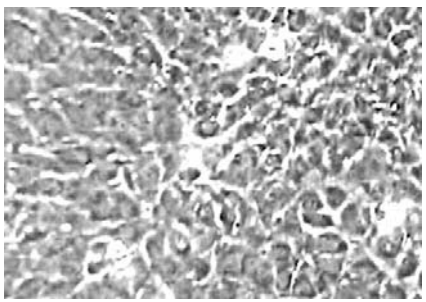
- Our goal:
  - Mimic the in vivo environment of the knee
  - Use an injectable polymer with two regions (bone and cartilage) that contains autologous cells encapsulated with growth factors

Production Process

Tissue Engineering

3D Matrix scaffold

Vascularization



Progenitor Cells

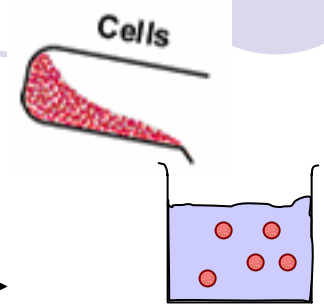
Growth Factors

Overview of Process

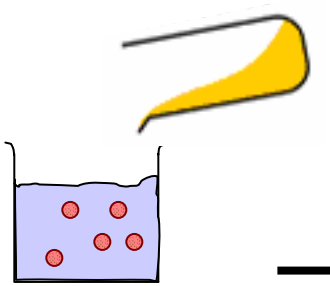
Cells harvested from patient

Cells Proliferated *in vitro*

Cells encapsulated in gelatin microparticles

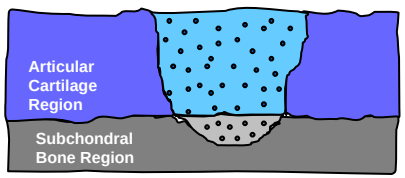


Cells and growth factors seeded into injectable polymer

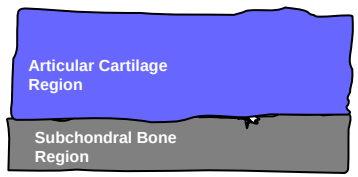


Polymer mixed with crosslinking reagent prior to injection

Polymer/cell suspension injected arthroscopically



Polymer crosslinks *in situ* forming hard scaffold



Over a few months scaffold degrades and cartilage regenerates

Harvesting/Proliferation of Chondrocytes

http://www.orthogastonia.com/patient_ed/html_pages/knee/knee_cartilage_surgery.html

- Elapsed Time ~ 5 weeks

Biopsy of cartilage from non-loadbearing region

Biopsy cut into pieces <2mm and placed in culture flasks

Culture media containing Collagenase and DNase added

Flasks placed in incubator for 24 hrs.

Media strained with 70μm cell strainers

Elutate centrifuged to isolate chondrocytes

Cells resuspended in medium containing ascorbic acid and antibiotics

Cells centrifuged again

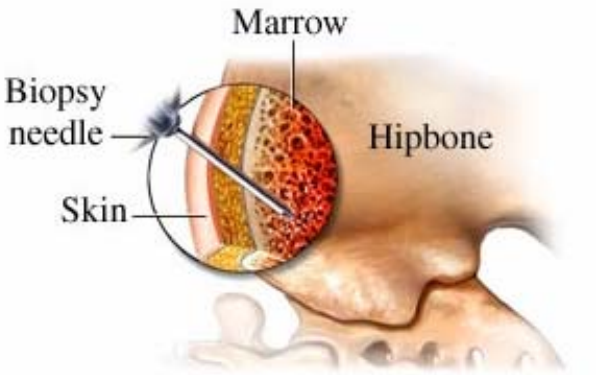
Cells resuspended in medium and cultured for 4 weeks at 37°C

Flasks washed with PBS and lifted with Trypsin

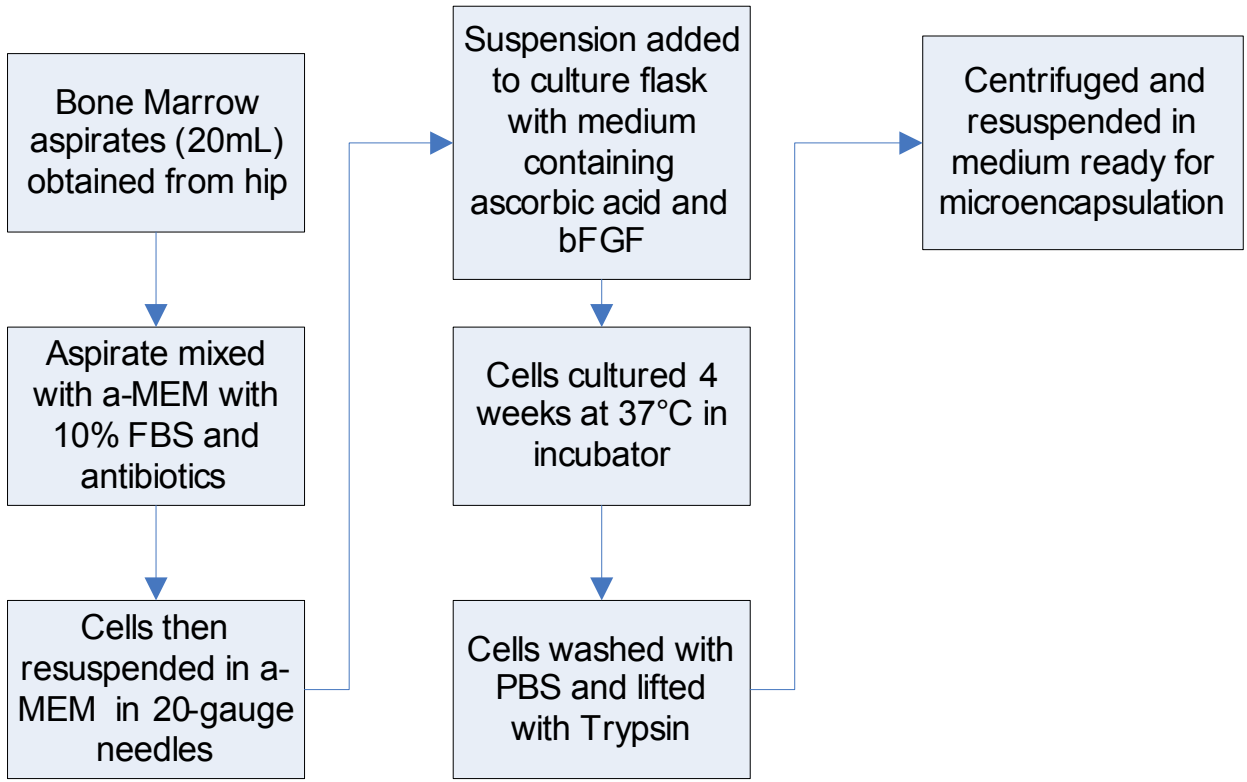
Centrifuged and resuspended in DMEM for microencapsulation

Harvesting/Proliferation of BMSC's

- Elapsed Time ~ 5 weeks
- Concurrently with Chondrocyte culture

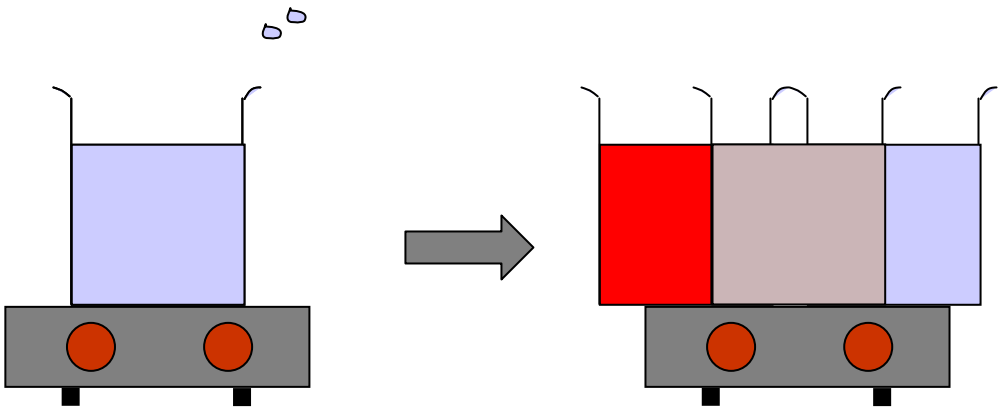


http://my.webmd.com/hw/health_guide_atoz/hw200221.asp



Preparation of Gelatin Solution

•Elapsed Time ~ 2 hrs



http://www.macbicnj.com/corning/90307.htm

30% (w/v) porcine gelatin solution in ddH_2O prepared

30g gelatin added to 100mL water 0.25 g at a time

Solution allowed to solidify at R.T. then stored at 4ºC till later use

An 11% (w/v) solution is then made from the 30% solution

DMEM and 30% Gelatin solution warmed to 50ºC

33.33g Gelatin and 56.67 mL DMEM added to beaker

pH adjusted to 7.2 with either NaOH or HCl

Resulting solution is sterilized by vacuum filtration in Laminar Flow Hood into a sterile media bottle

Stored at 4ºC till Microparticle formation

Encapsulation and Surface Crosslinking

45mL of 11% gelatin warmed to 37ºC and diluted with 5mL sterile suspension of cells in DMEM

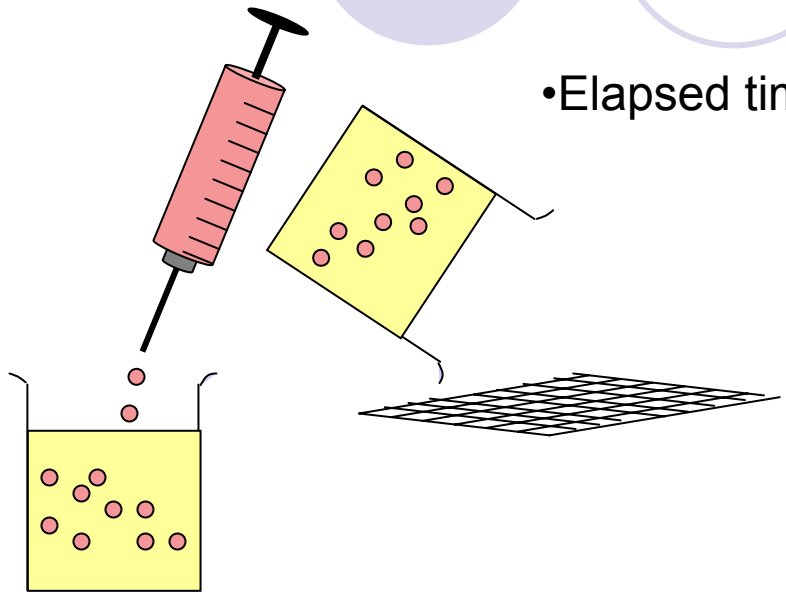
Gel/cell solution placed in sterile 30mL syringes with 22 gauge needles

Allowed to cool to 34ºC

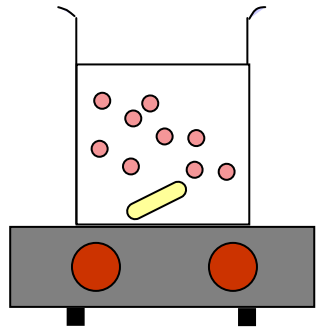
400mL sterile mineral oil with 5mM DSP (crosslinks on surface) prepared at 10ºC

Solution then dripped into oil to form microparticles containing cells

Causes microparticles to form due to hydrophobic interactions and harden due to temperature change



•Elapsed time ~ 2 hrs

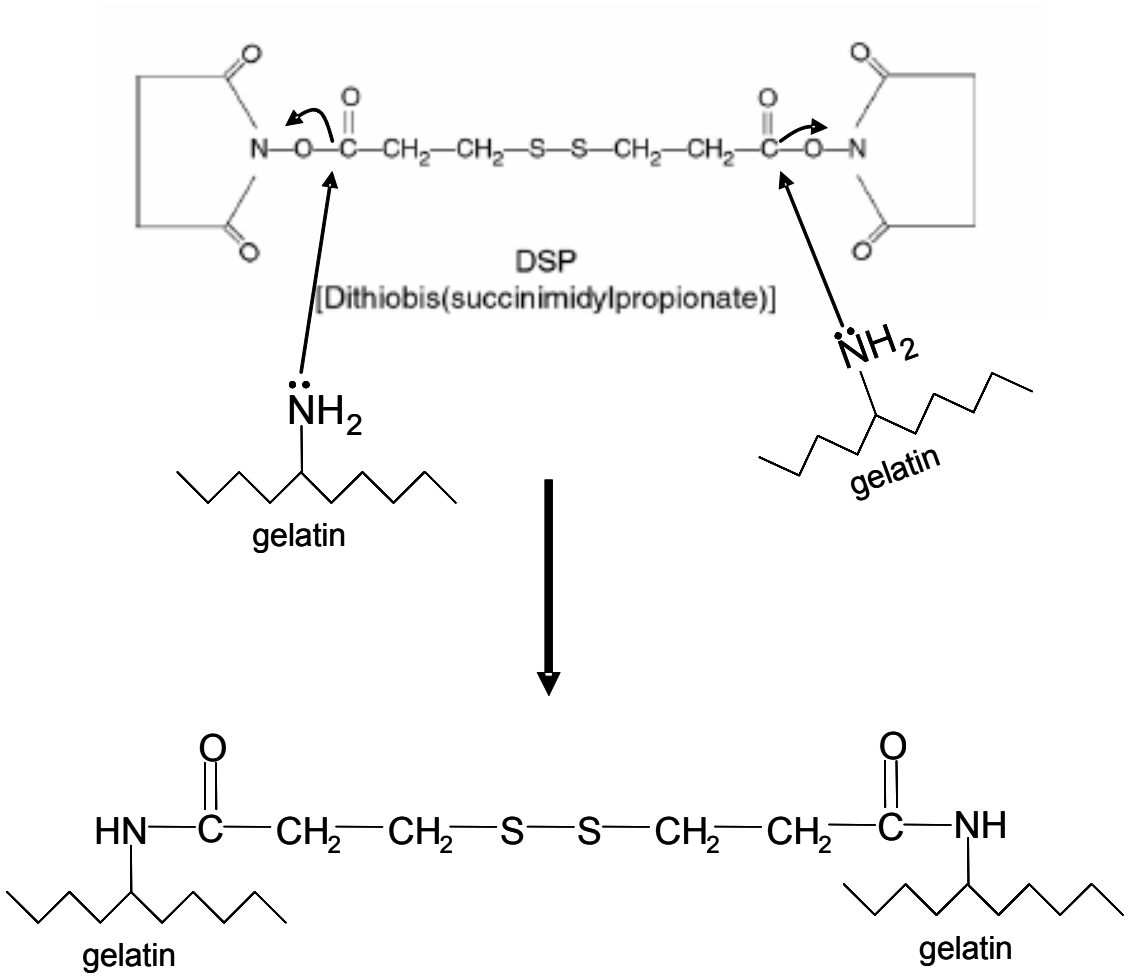


Microparticle solution filtered on 80µm meshes

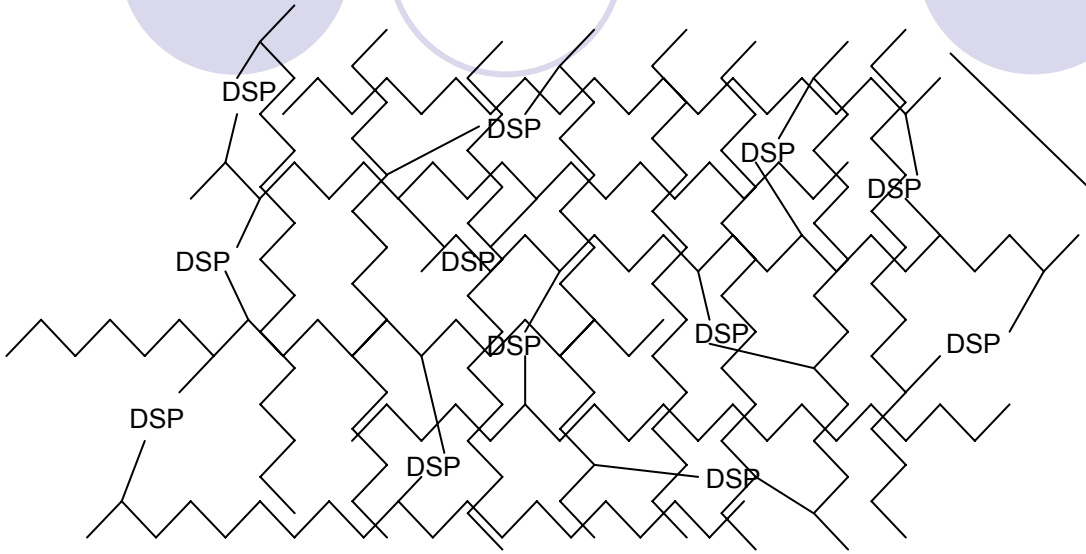
Transferred to PBS at 15ºC agitated 2 min

Microparticles filtered again and placed on absorbent towels in ice bath

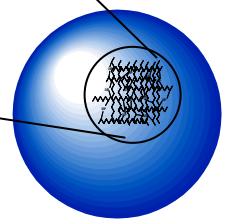
Microparticle Chemistry



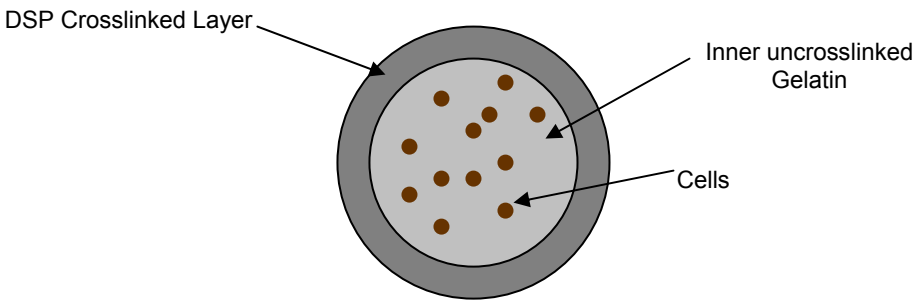
Crosslinking at the Surface



View at Microparticle Surface



Gelatin Microparticle Containing Cells



Cross-Section of Microparticle

Gelatin Microparticles

- This membrane will help maintain the mechanical integrity for a short time
- Prevents enzymatic degradation mainly by steric hindrance
- Prevents reverse thermal gelation of the particle by holding the gelatin molecules together

Liang et al. Genipin-crosslinked gelatin microspheres as a drug carrier for intramuscular administration: *In vitro* and *in vivo* studies J Biomed Mater Res 65A: 271–282, 2003

Preparation of PPF

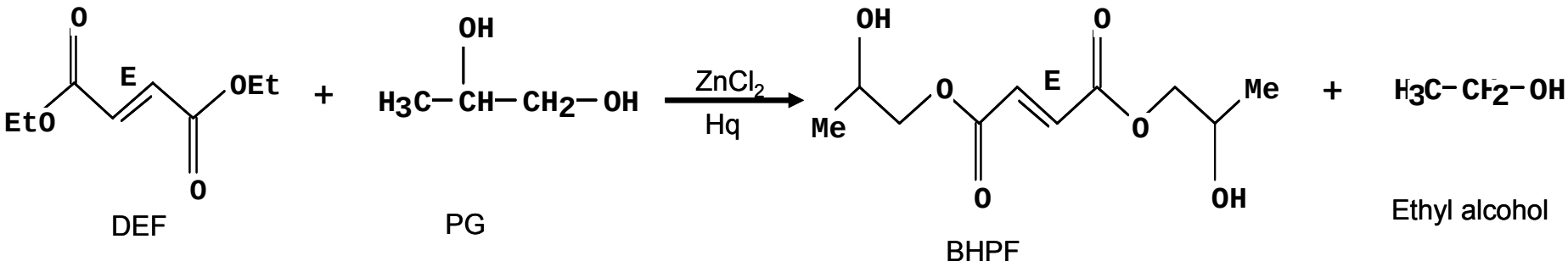
- Poly(propylene fumarate) is prepared in a two step reaction scheme:
  - First is formation of a diester
  - Second is a transesterification reaction
  - PPF will be prepared with both β-TCP particles and as a copolymer with poly(ethylene glycol)

Payne, R.G. et al. *Development of an injectable, in situ crosslinkable, degradeable polymeric carrier for osteogenic cell populations, Part 2*. Biomaterials 23: 4373-4380, 2002.

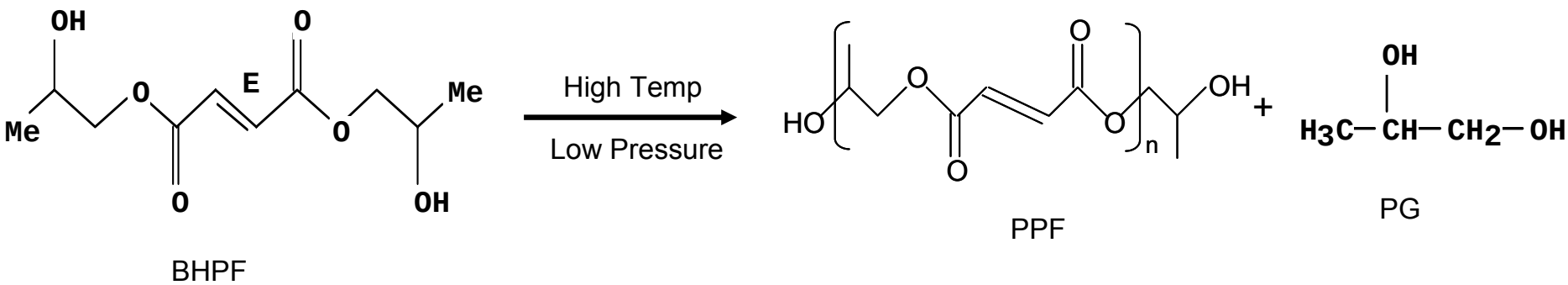
Polymer Chemistry

- The reactions involved in the production of PPF are as follows:

Step 1 – formation of Diester



Step 2 – transesterification



Microparticle Seeding and Injection Preparation

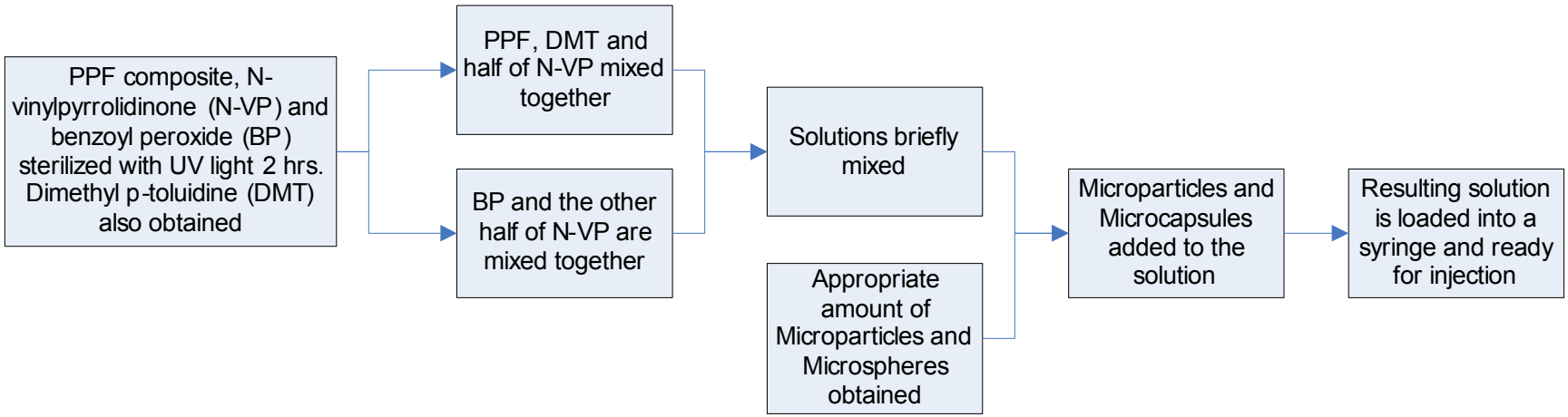


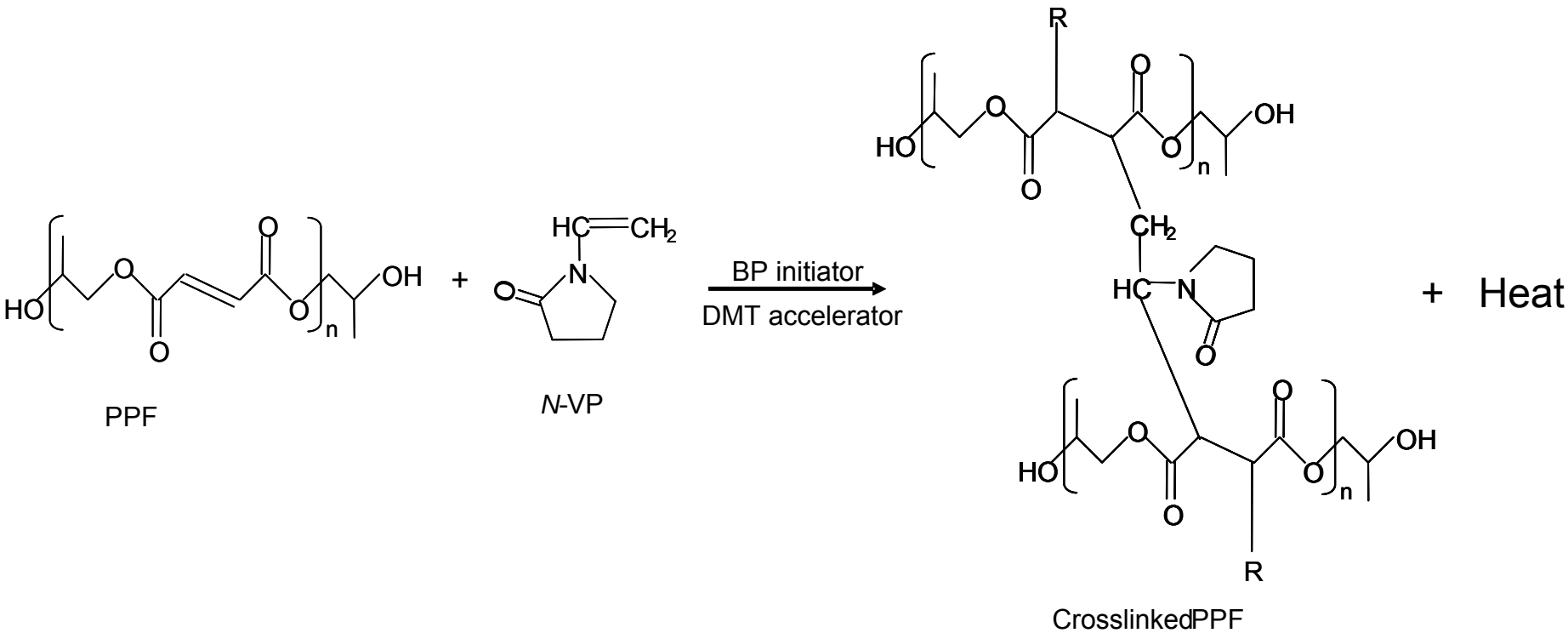
Table 1. Relative Amount of each Component in Crosslinking Reaction

Component	Amount
PPF/β-TCP or PPF-co-EG	1.0 g
N-VP	0.1 g
Benzoyl Peroxide	0.0015 g
DMT	2.5 µL

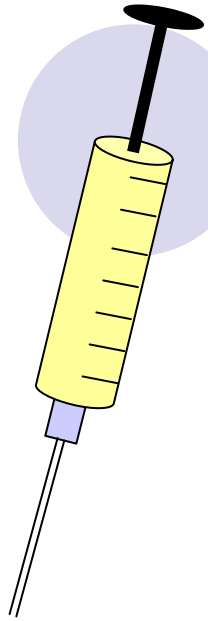
- This procedure is performed directly before injection

Polymer Chemistry

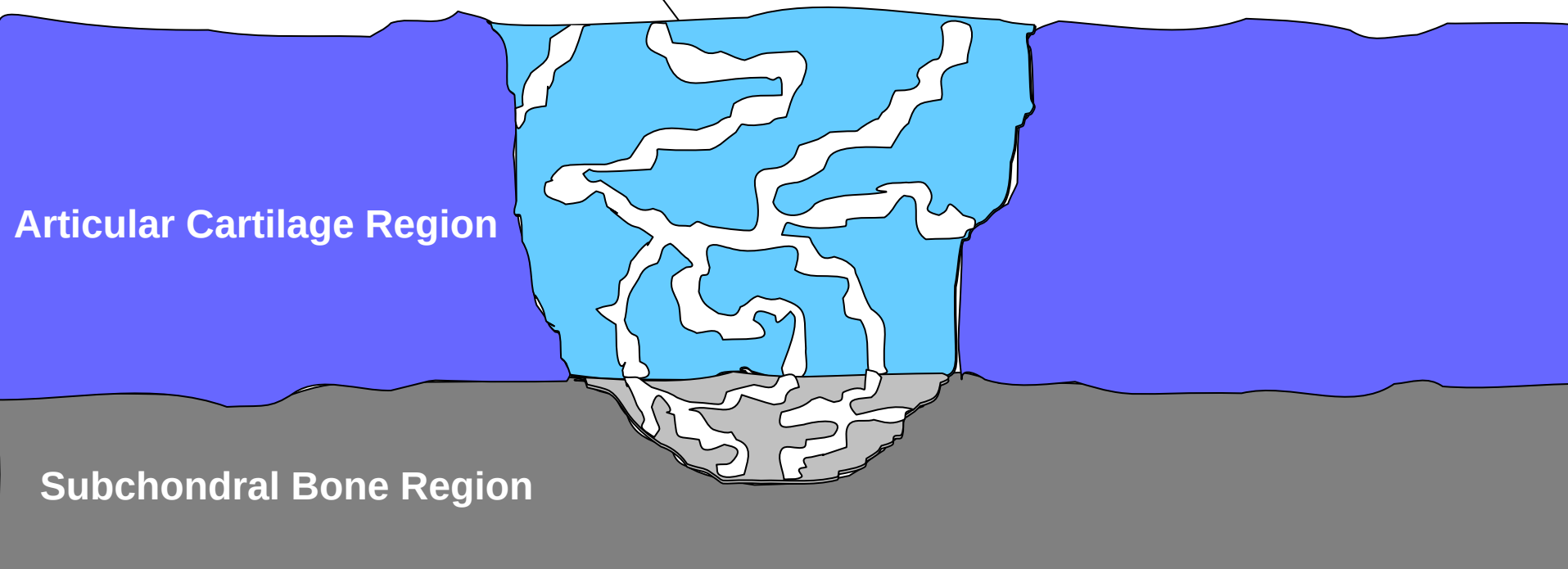
- The PPF is crosslinked *in situ* using N-VP and benzoyl peroxide as an initiator, and DMT as an accelerator



Close-up of Injection Procedure



Gelatin Microparticles containing cells then disperse approximately 1 hr. post injection at 37ºC and pores are created also securing material into place

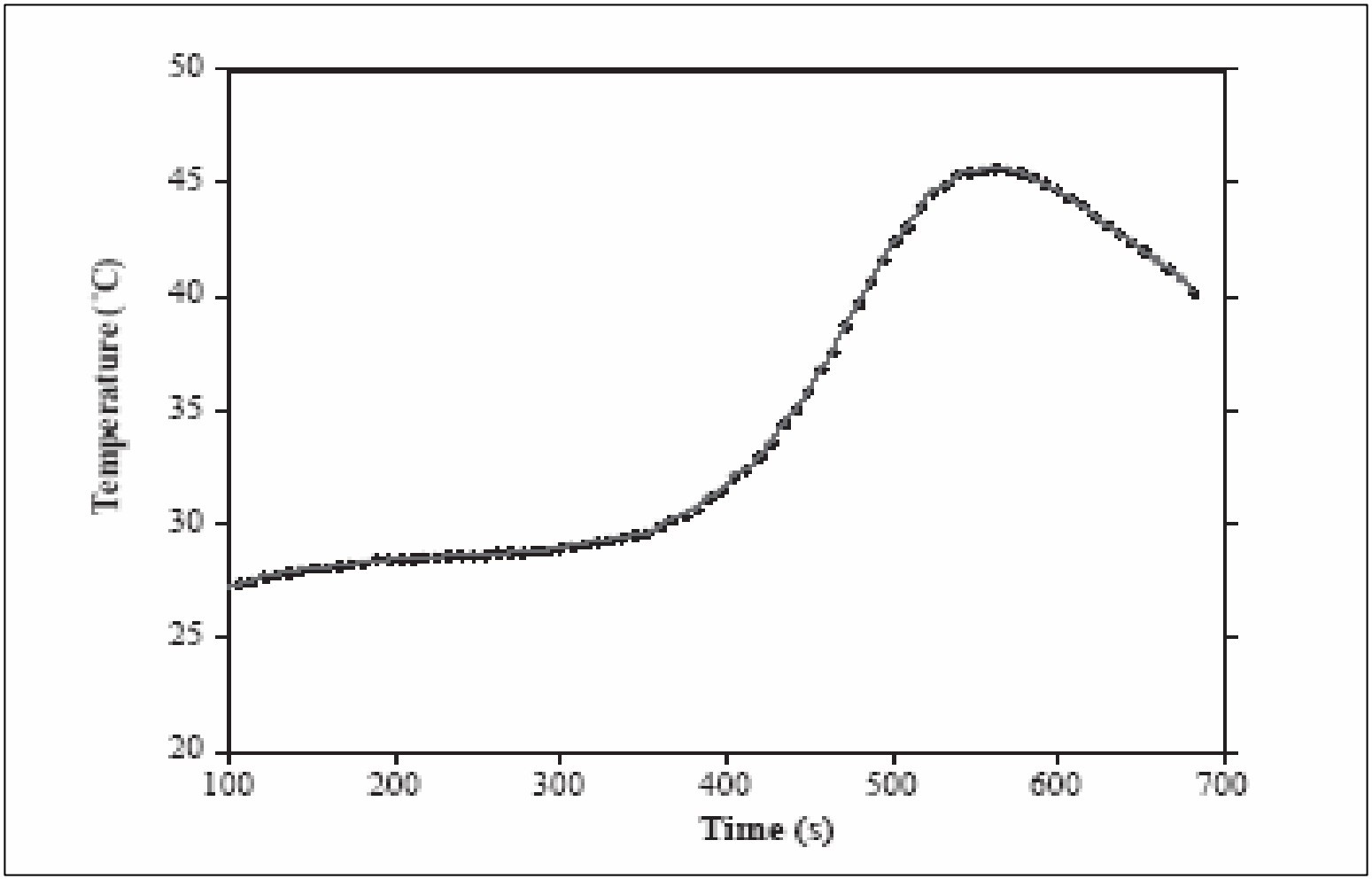


Temperature Profile

Polymer Crosslinking Reaction

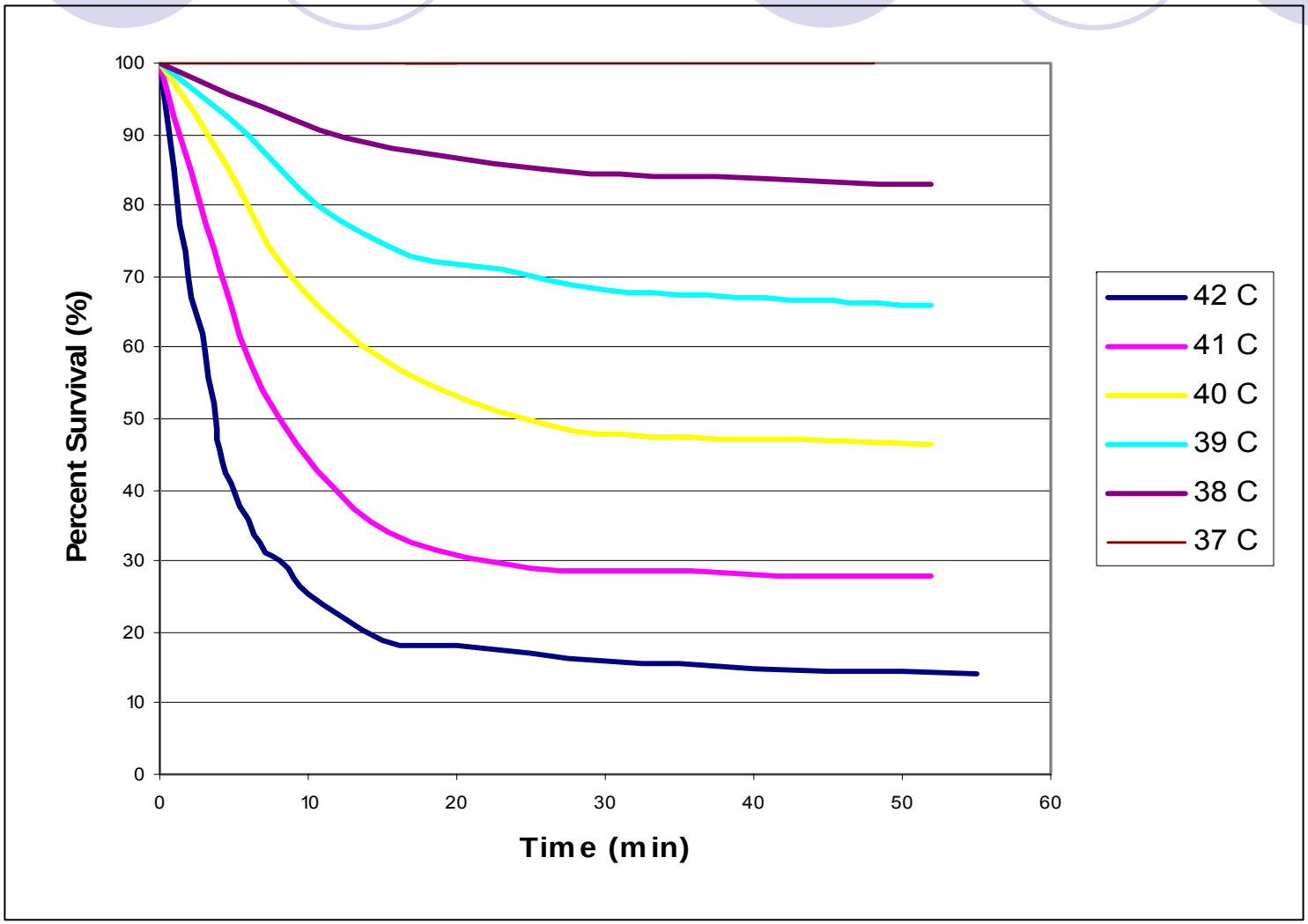
- Heat released during cross-linking reaction
- At increased temperatures, gelatin undergoes reverse thermal gelation
  - Gelatin becomes fluid
  - Cell survival decreases
- DSP cross-linking on surface of microcapsules in order to maintain mechanical integrity

Temperature Profile of Polymer Crosslinking Reaction



Payne, R.G. et al. Development of an injectable, in situ crosslinkable, degradeable polymeric carrier for osteogenic cell populations, Part 2. *Biomaterials* 23: 4373-4380, 2002.

Cell Survival vs. Temperature

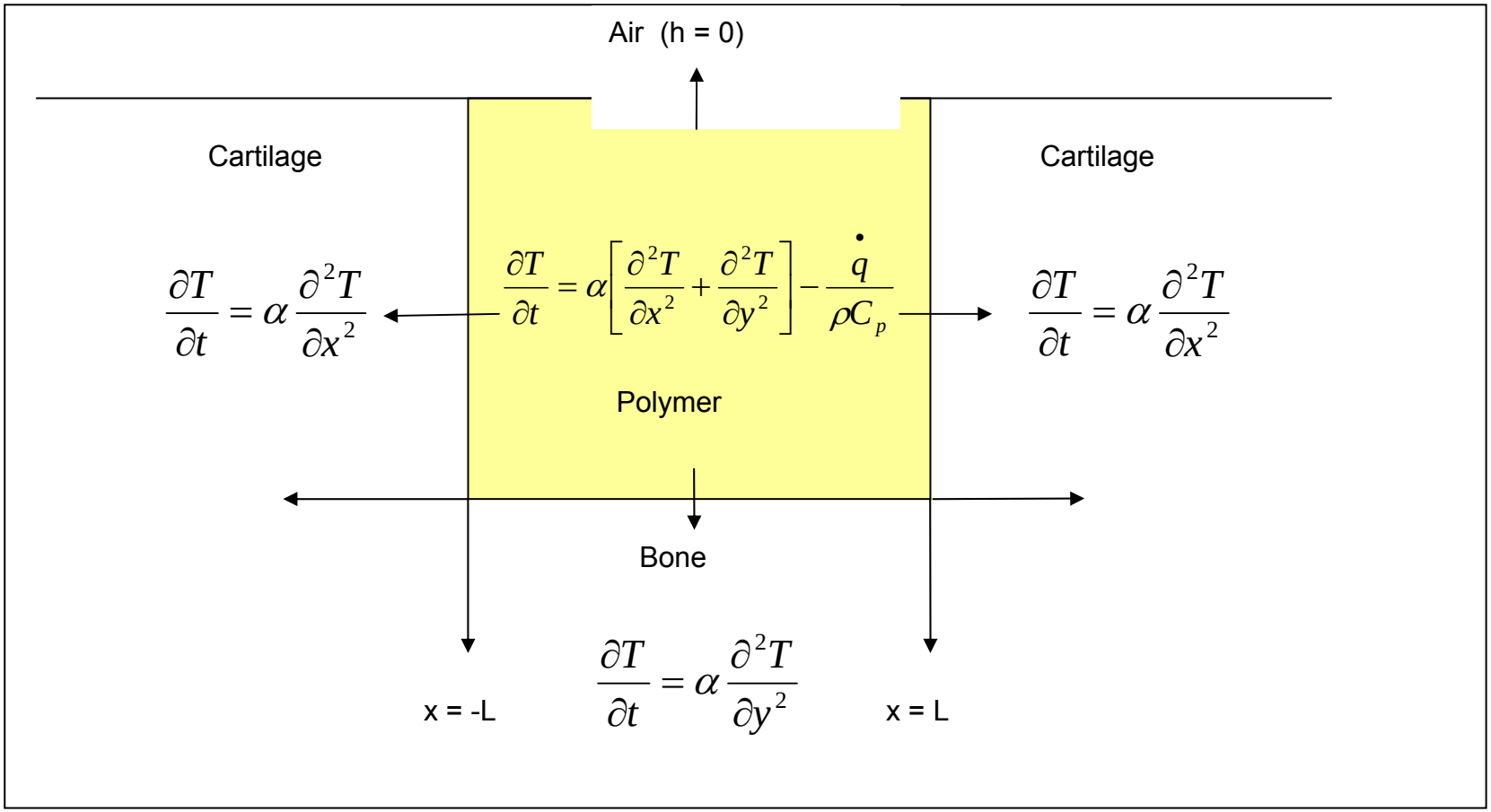


Lin et al. Stability of Heating Temperature on Cytotoxicity. *Int. J Radiation Oncology Biol. Phys.* Vol. 13, pp. 1869-1873. 1987.

Temperature Profiles

- Maximum temperature of polymer = 45.7ºC after 562 s (9.4 min)
- Polymer is above critical temperature of 37ºC for less than 5 minutes
- Model behavior of temperature rise within injection as a function of time in order to determine cell viability
- Model can determine optimal injection temperature

Mathematical Model



Assumptions

- Boundary Conditions:

$$\alpha_u \left.\frac{\partial T}{\partial x}\right|_{x=L,t} = \alpha_T \left.\frac{\partial T}{\partial z}\right|_{z=0,t}$$

$$T(x,0) = T_o$$

- From symmetry: $T_x(L,t) = T_x(-L,t)$
- One-dimensional inside term:

$$\alpha \frac{\partial T}{\partial t} = \frac{\partial^2 T}{\partial x^2} + q(t)$$

- Heat generation term: $q(t) = 50e^{-214.75t}$

Inside Term

- From separation of variables:

$$T(x,t) = C(t) + Dx + \sum A_n(t)\sin\lambda_n x + \sum B_n(t)\cos\lambda_n x$$

- Using Fourier series and rules of orthogonality:

$$T(x,t) = \left(K - \frac{k_1}{\alpha k_2} e^{-k_2 t} \right) + \sum 2 \left[\frac{u_o - K + \left(k_1 / k_2 \right)\alpha}{L\lambda_n} \right] e^{\left(-\lambda^2/\alpha\right)t} \sin\lambda_n x$$

- Unknown K is determined from outside equation

Outside Term

- In cartilage region in one dimension:

$$\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2}$$

- Taking the Laplace transform:

$$\hat{T} = \cancel{A e^{\sqrt{\frac{s}{\alpha}} z}} + B e^{-\sqrt{\frac{s}{\alpha}} z}$$

- From boundary conditions:

$$\hat{T}(z,s) = F(s) \cdot G(s) \text{ where } F(s) = T(L,s) \text{ and } G(s) = e^{-\sqrt{\frac{s}{\alpha}} z}$$

Initial Solution

- By using the initial boundary condition:

$$T_{out}(0,s) = T_{in}(L,s)$$

- From the theory of convolution:

$$T(z,t) = f(t) * g(t) = \int_0^t f(t-\xi) g(\xi) d\xi$$

- Therefore the final equation for the outside term:

$$\left[-2\frac{K}{\sqrt{\Pi}} erfc\left(\frac{z}{2\alpha t}\right)\right] - \left[\frac{k_1}{\alpha k_2 \sqrt{\Pi}} e^{-k_2 t}\left(\frac{\alpha t}{2z}\right)\left(1 + 2e^{\frac{k_2 t}{4}} + 2e^{\frac{k_2 t}{2}} + 2e^{\frac{3k_2 t}{4}} + e^{k_2 t}\right)\right]$$

Inside vs. Outside

- By using boundary condition $T_{out}(0,t) = T_{in}(L,t)$

$$T(L,t) = \left(K - \frac{k_1}{\alpha k_2} e^{-k_2 t} \right)$$

$$T_{out}(0,t) = -2\frac{K}{\sqrt{\Pi}}$$

- These two equations are used to solve for the unknown constant K

$$K = \frac{k_1}{\left(1 + \frac{2}{\sqrt{\Pi}}\right)\alpha k_2} e^{-k_2 t}$$

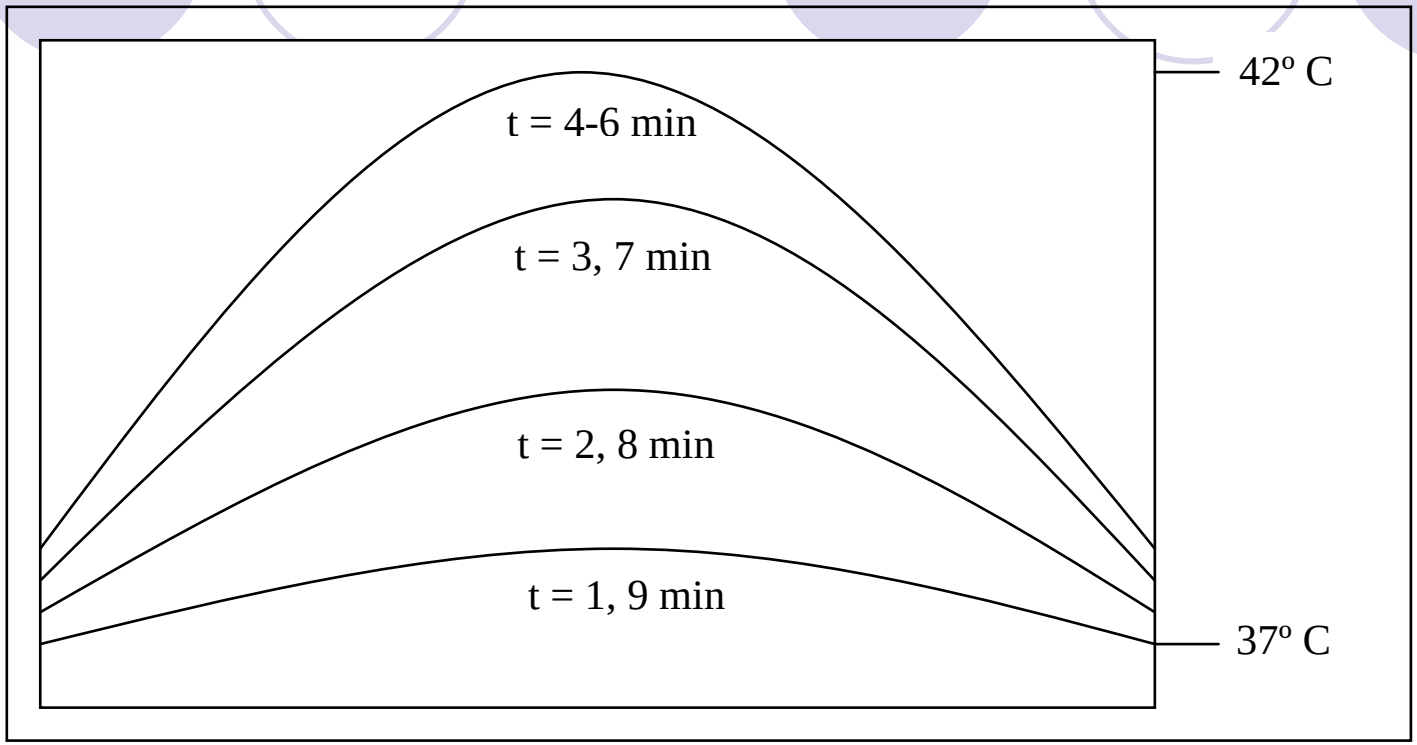
Final Solution

- The temperature flux is:

$$\frac{\partial T}{\partial x} = \frac{2\left(T_o - \frac{k_1}{\left(1 + \frac{2}{\sqrt{\Pi}}\right)\alpha k_2} e^{-k_2 t} + \left(k_1 / k_2\right)\alpha \right)}{L} \sum e^{\left(\lambda_n^2/\alpha\right)t} \cos\lambda_n x$$

- All constants known
  - α is thermal diffusivity of water (cartilage is 80% water)
  - L is 0.035 cm (average size of defects)

Expected Behavior



Based on the internal body temperature of 37 ºC and the temperature rise to 42 ºC due to polymerization, the expected temperature behavior is plotted.

Future Work

- Inside term to be solved in two dimensions
  - Cylindrical coordinates may be simpler
- Graphical representation of temperature profile during cross-linking reaction
- Microparticle temperature profile determined to analyze cell survival with given sizes

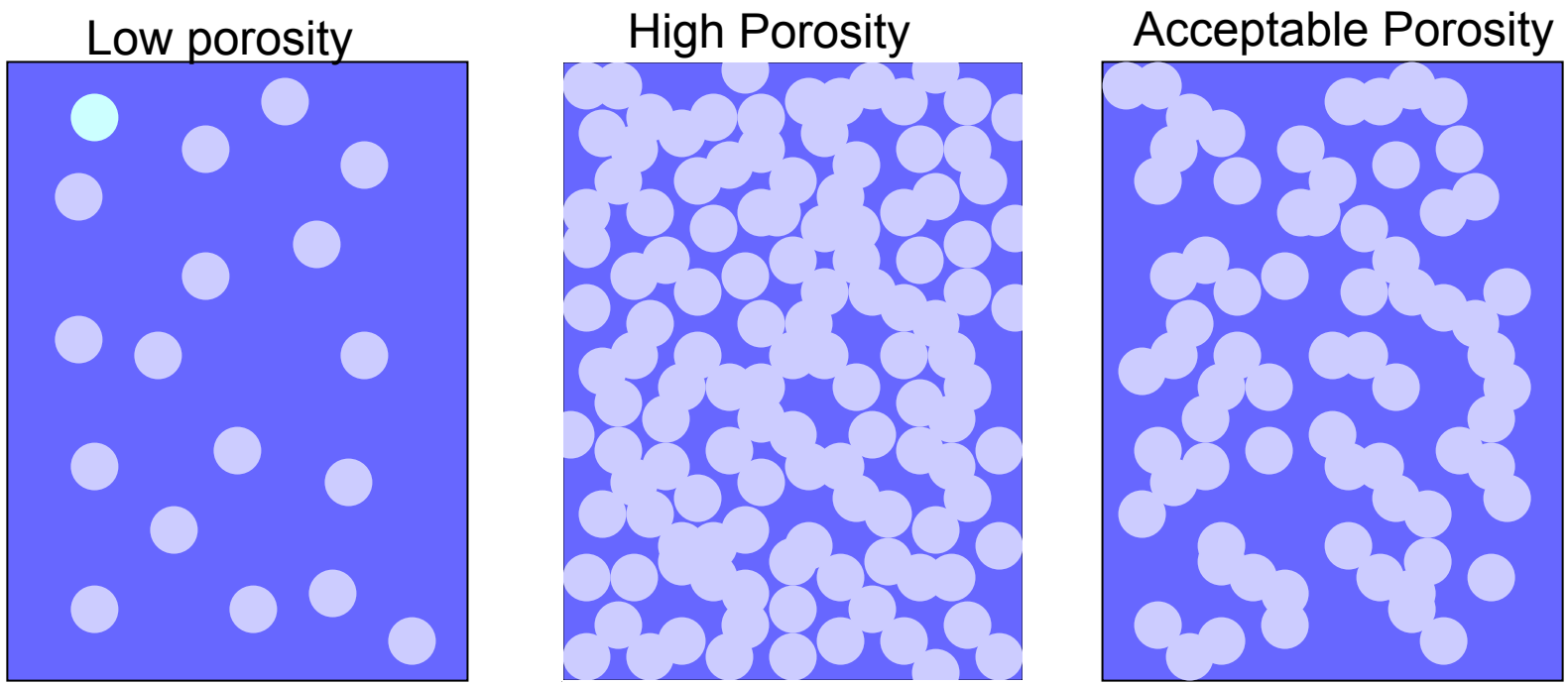
Porosity

Porosity

- a measure of the voids *or*
- ratio of volume of openings/ total volume of the material
- Increasing porosity will
  - Allow greater amount of cells to be injected
  - Decrease mechanical stability

Networking

- Creating Pores within the Polymer Structure



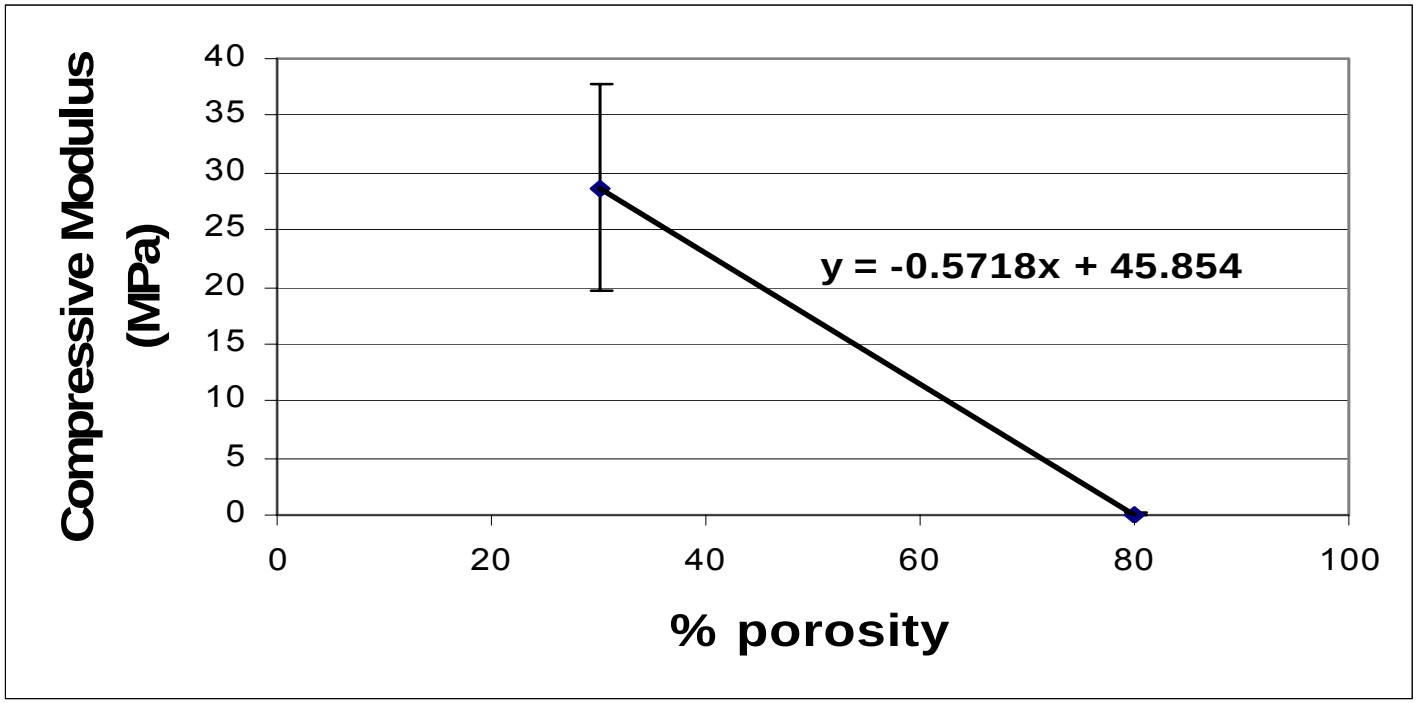
Cartilage Modeling

- Compressive modulus: 0.4 – 1.5 MPa

Mechanical Properties of PPF

- 30 % porosity
  - Compressive modulus: 28.7 ± 9.1 MPa
- 80% porosity
  - Compressive modulus: 0.11± 0.02 MPa

Mechanical Properties of PPF



(Assuming linear relationship)

Porosity range: **77**-75%

Amount of Microcapsules Needed

- After polymerization, microcapsules form the void space
- Example: 77% porosity
  - 77 volume % microparticles
  - 23 volume % PPF/β-TCP or PPF-co-EG

FDA Approval Process

Regulation and Classification

- CDRH (Center for Devices and Radiological Health)
  - Medical Device
- Class III
  - Implant lacks safety and effectiveness support
  - Risky side effects of implant
  - Pre-Market Approval

Pre-Market Approval

- Modular PMA
- Non-clinical studies
  - Laboratory experiments
  - Production of materials
  - Animal testing
- Clinical studies
  - Voluntary patients

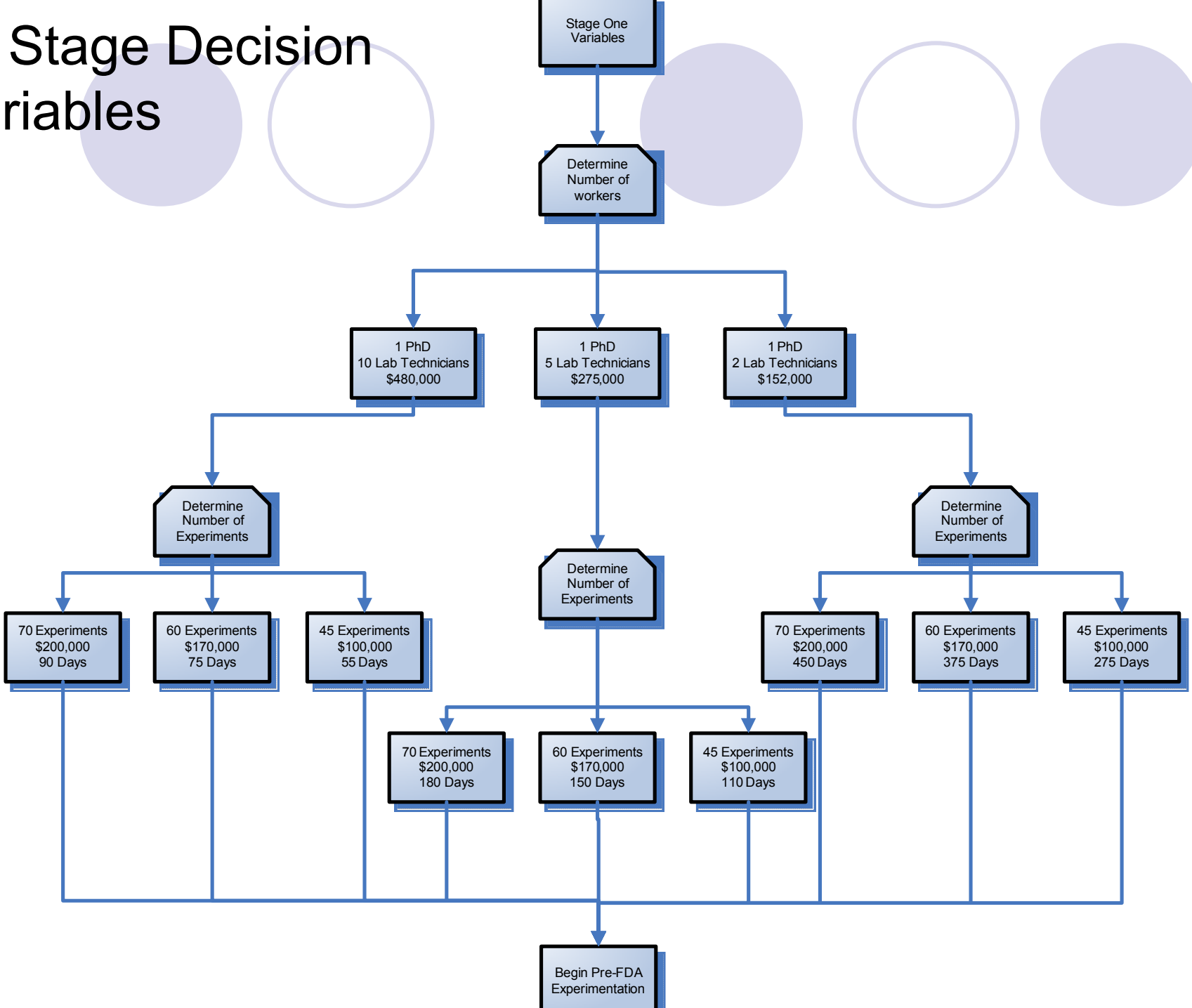
FDA Approval Modeling

- Uses two-stage stochastic modeling
  - 1st stage decision variables
    - “Here and Now” decisions
    - Number of laboratory technicians and experiments
  - 2nd stage decision variables
    - Made after an outcome
    - Whether to continue or not post failure

1st Stage Decision Variables

- 10, 5, or 2 laboratory technicians
  - Affect cost, time, but not probability
- 70, 60, or 45 pre-FDA experiments
  - Affect cost, time, and probability
  - More experiments provides for greater probability of success

1st Stage Decision Variables



Pre-FDA Experiments

- 45 experiment decision
  - This set will contain the base number and type of experiments, deemed minimally essential prior to PMA filing and are as follows:
    1. Synthesis of Poly(propylene fumarate)
    2. Gelatin Microencapsulation Histology
    3. Evaluation of cell growth on polymer
    4. Growth Factor encapsulation and effect
    5. Basic biocompatibility tests

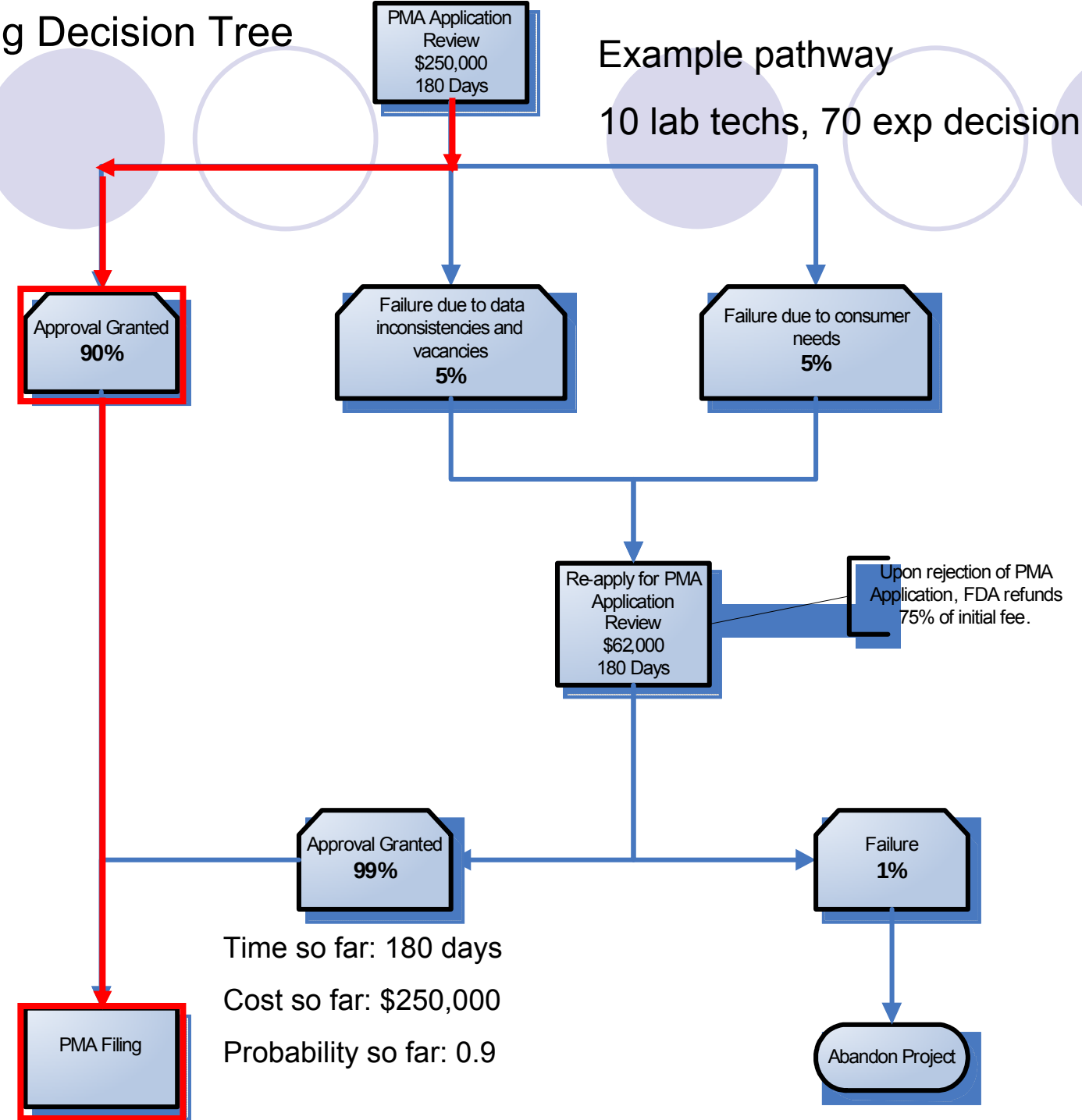
Pre-FDA Experiments

- 60 experiment decision
  - In addition to the aforementioned experimental sets, 3 more additional experiment types will be performed for the 60 experiment decision, and are as follows:
    6. Evaluation of polymer mechanical properties pre- and post-implantation
    7. Evaluate the optimal cell seeding density of the gelatin microparticles
    8. Evaluate optimal growth factor and cell density ratio within the construct

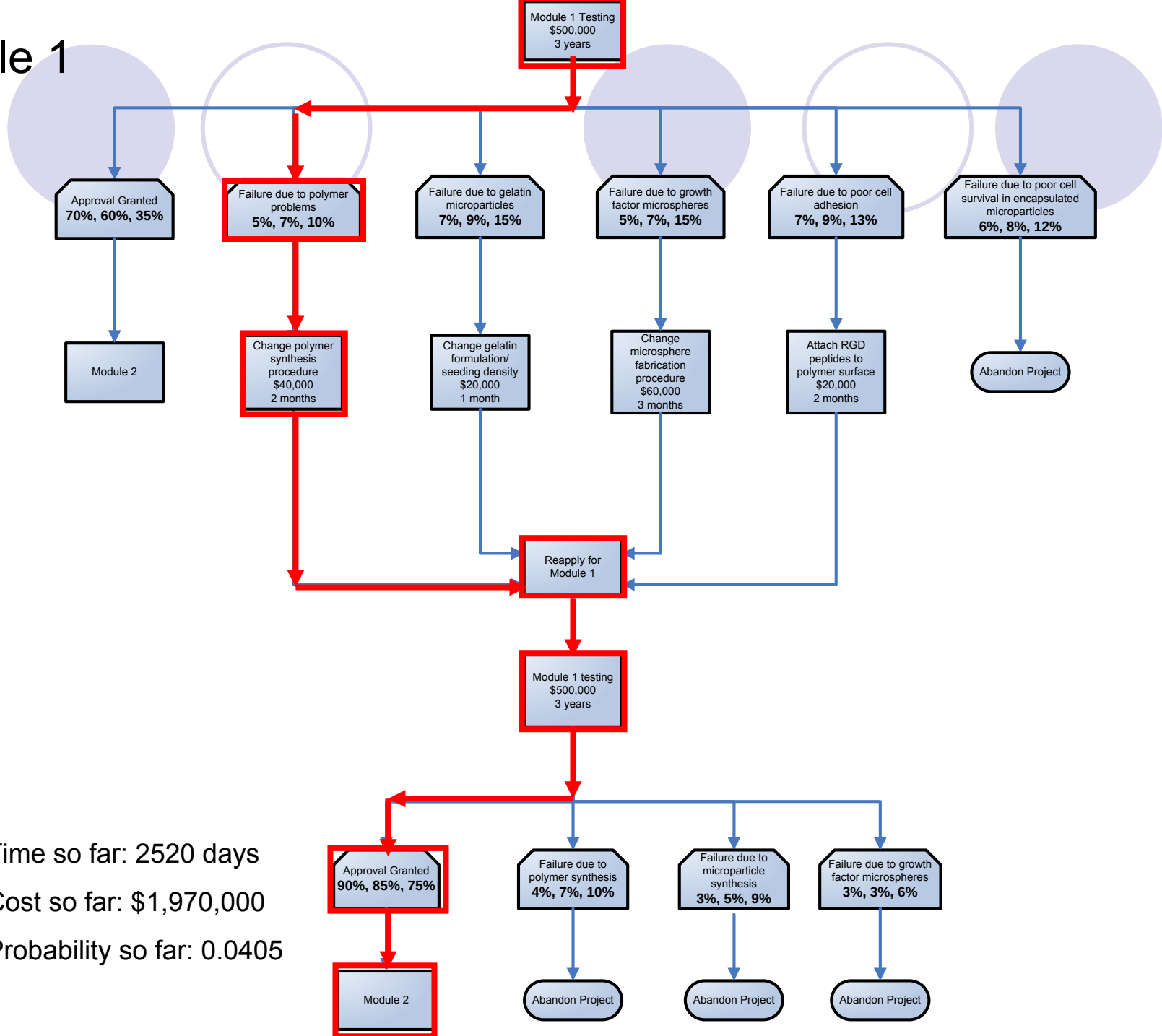
Pre-FDA Experiments

- 70 experiment decision
  - In addition to all the previously described experiments for 45 and 60, 2 additional experiment types will be performed as follows:
    9. Evaluate the degradation rate of the polymer versus the cell tissue ingrowth
    10. Evaluate the longterm success rate of procedure on white rats

PMA Filing Decision Tree



Module 1

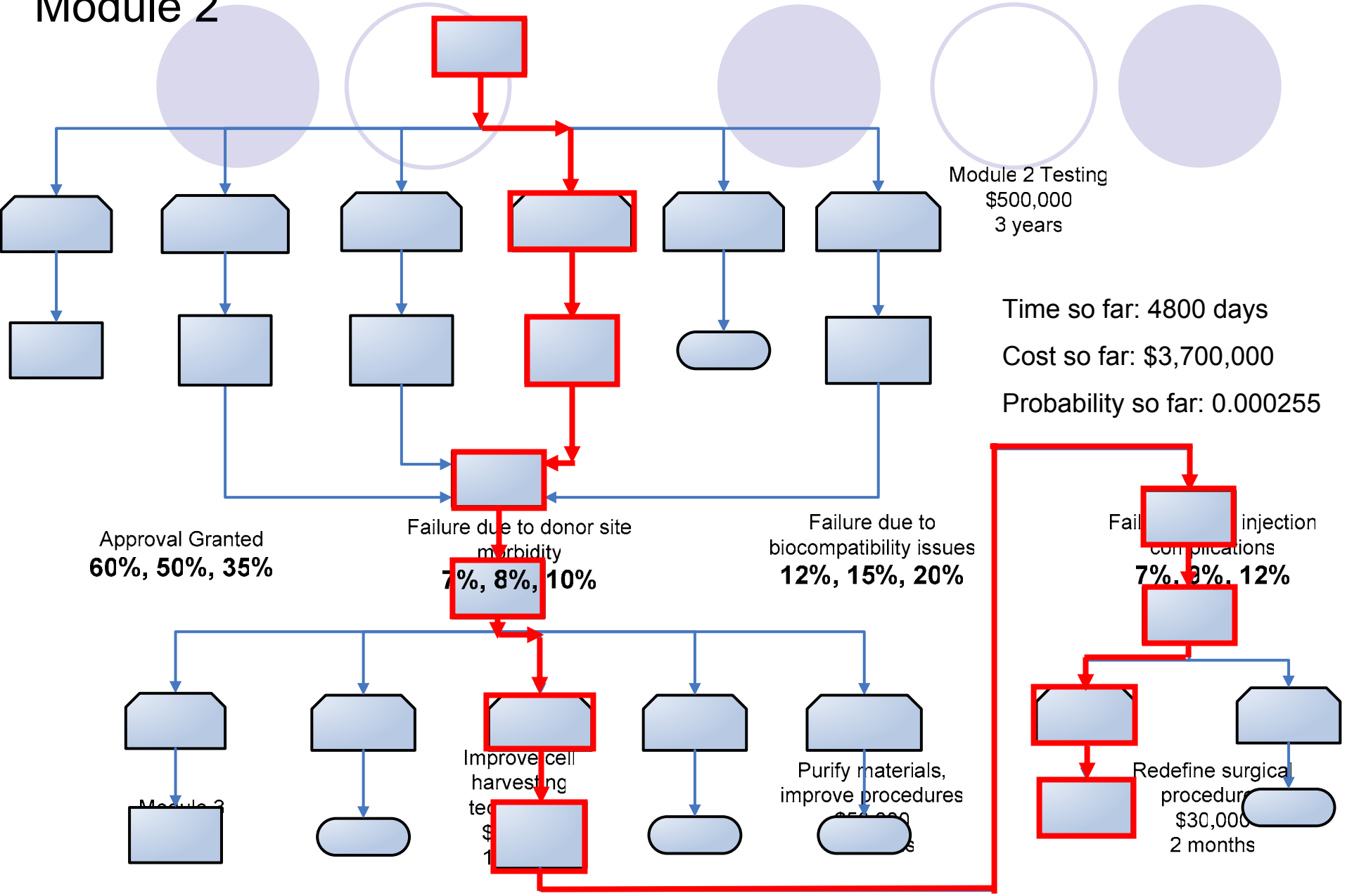


Time so far: 2520 days

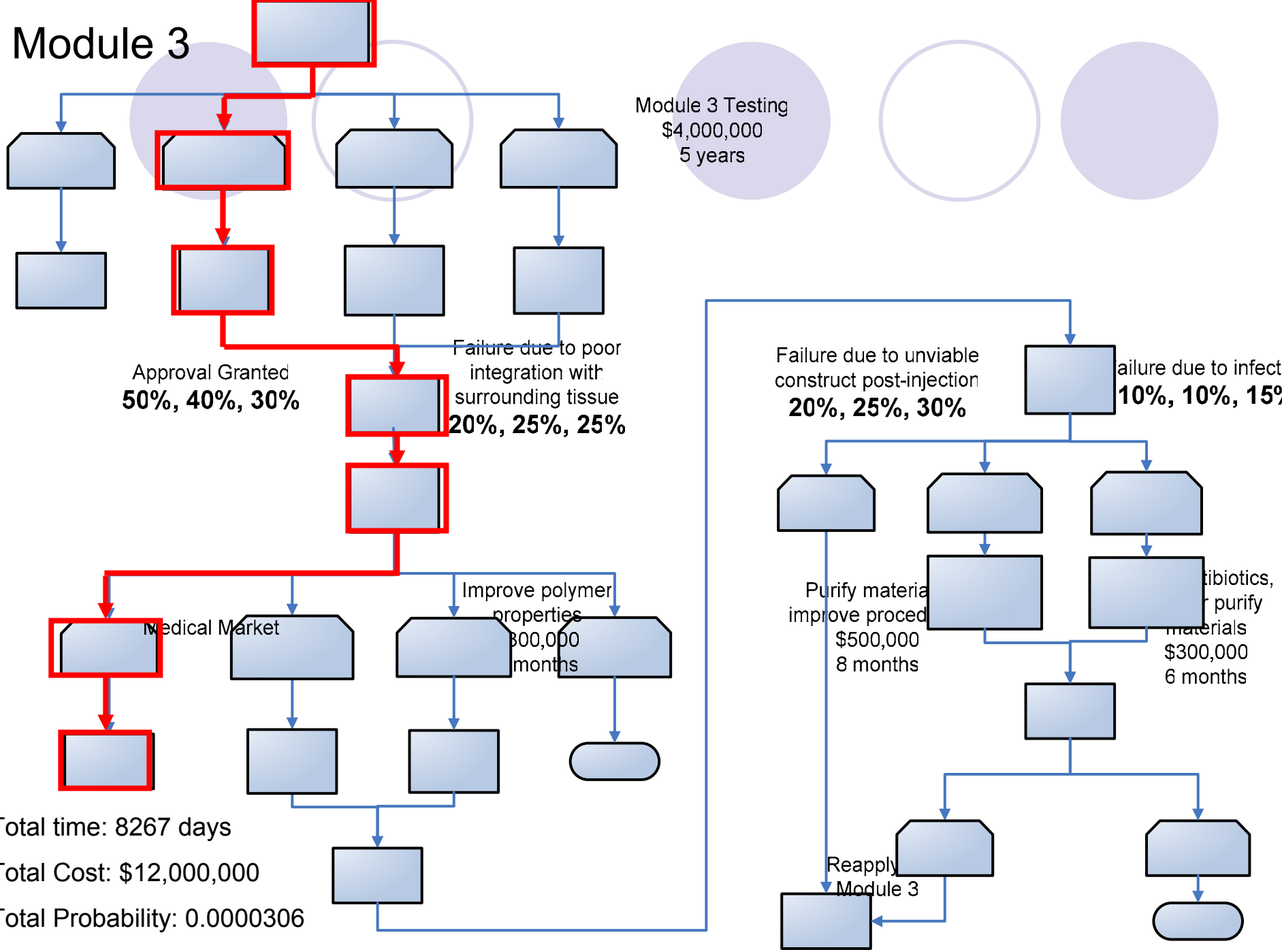
Cost so far: $1,970,000

Probability so far: 0.0405

Module 2


Module 2 Testing
$500,000
3 years
Time so far: 4800 days
Cost so far: $3,700,000
Probability so far: 0.000255
Approval Granted
60%, 50%, 35%
Failure due to donor site morbidity
7%, 8%, 10%
Failure due to biocompatibility issues
12%, 15%, 20%
injection
7%, 12%
Improve cell harvesting
Purify materials, improve procedures
Redefine surgical
$30,000
2 months

Module 3



Total time: 8267 days

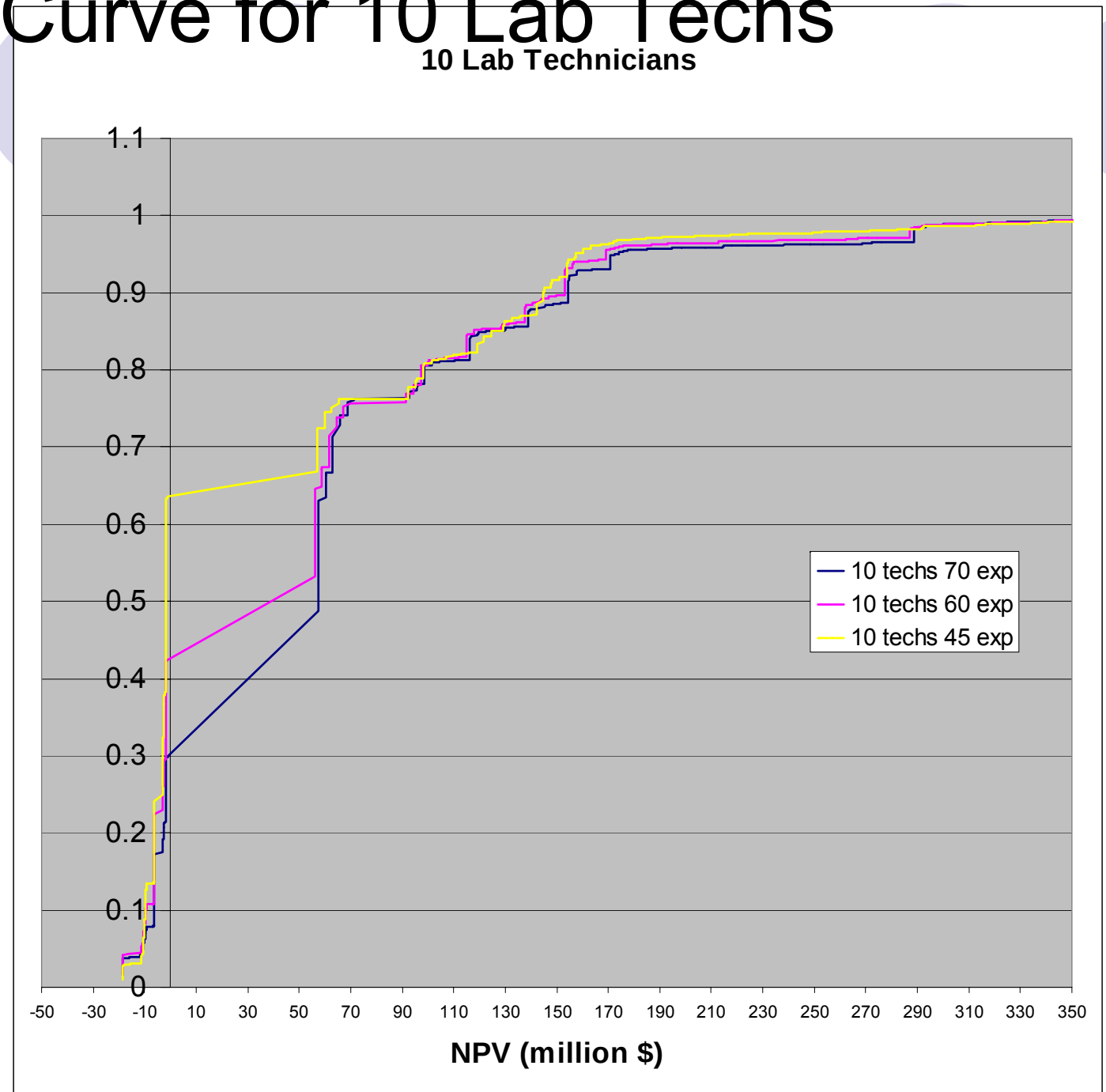
Total Cost: $12,000,000

Total Probability: 0.0000306

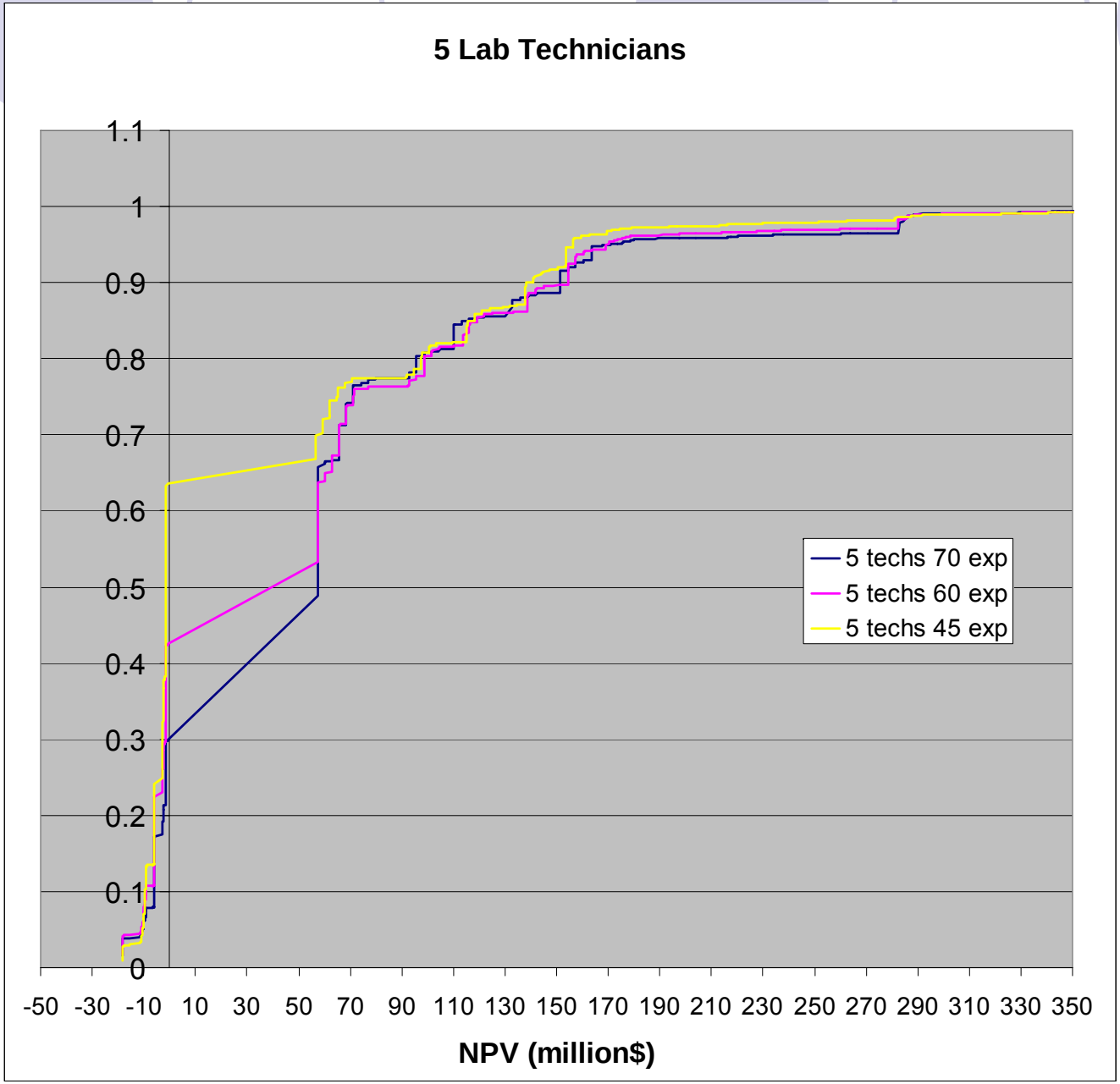
FDA Approval Modeling

- Number of paths possible
  - 5,290 different paths possible through the decision trees for each first stage decision variable (47,610 total pathways)
  - 2,970 lead to success and 2,320 lead to failure

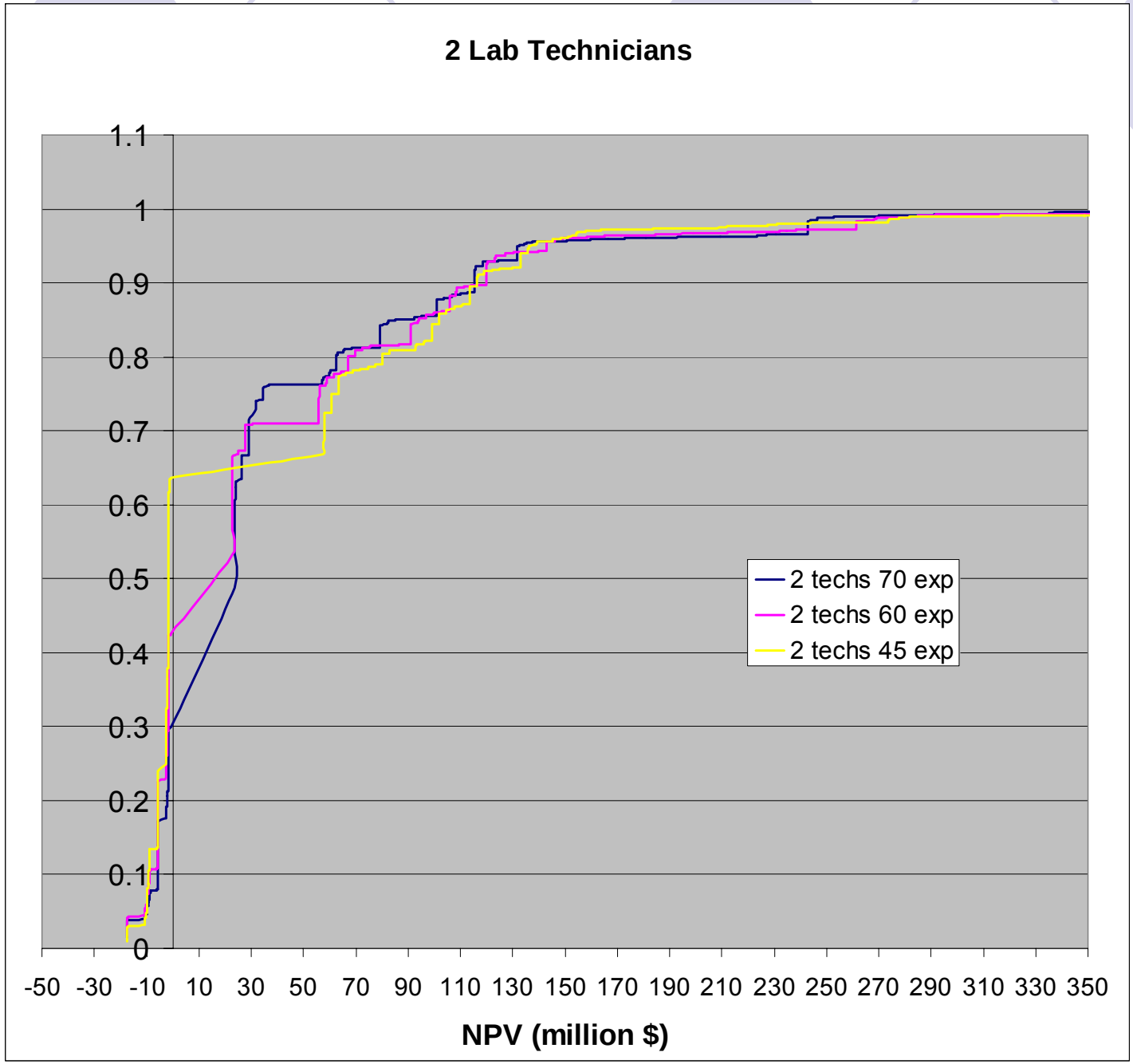
Risk Curve for 10 Lab Techs



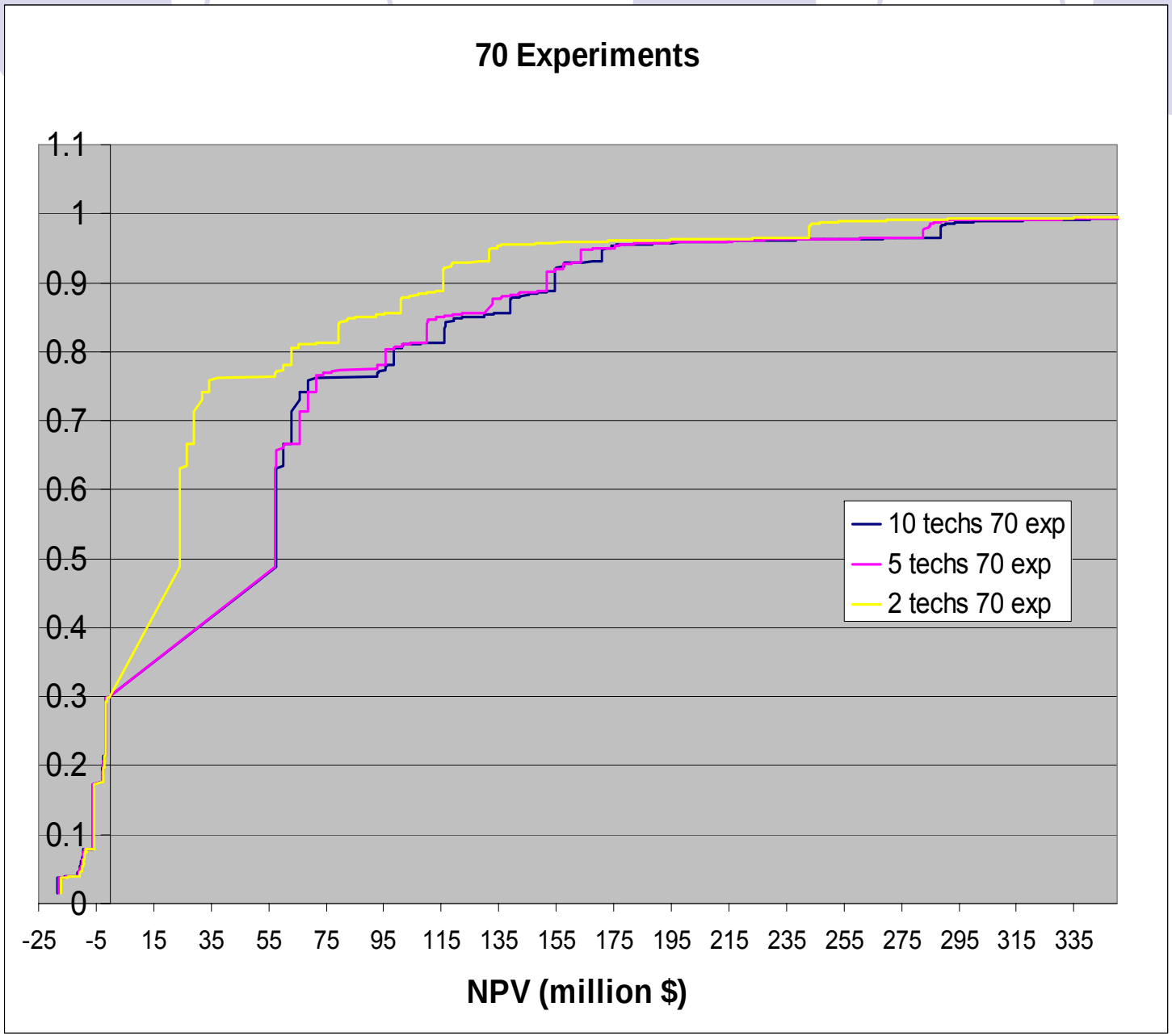
Risk Curve for 5 Lab Techs



Risk Curve for 2 Lab Techs



Comparison at 70 Experiments



FDA Approval Modeling

- Shortest path
  - Path in which approval is met with no failures using 10 lab techs and 45 experiments
    - Total Time: 4250 days or 11.6 years
    - $ENPV_{cost}$: $4.4 million
    - ENPV: $1130.4 million
- Cheapest Path
  - Path in which approval is met with no failures using 2 lab technicians and 45 experiments
    - Total Time: 4470 days or 12.2 years
    - $ENPV_{cost}$: $3.96 million
    - ENPV: $1012.8 million

FDA Approval Modeling

- Longest path
  - Path in which every failure possible is met using 2 lab techs and 70 experiments
    - Total Time: 11483.5 days or 31.5 years
    - $ENPV_{cost}$: $8.7 million
    - ENPV: -$8.7 million
- Most Expensive Path
  - Path in which every failure possible is met using 10 lab technicians and 70 experiments
    - Total Time: 9060 days or 24.8 years
    - $ENPV_{cost}$: $18.6 million
    - ENPV: -$18.6 million

Business Plan

Business Goals

- Treatment of cartilage deficiencies using autologous cultured chondrocytes
- FDA Approval
- Provide non-invasive surgery at a competitive cost to be covered by insurance
- Break even point after 2-3 years
- Expand company over time to increase profit and production

Business Plan

- Culture and grow cells in facilities
- Ship materials and cultured cells to hospitals
- Train surgeons on handling and preparation of scaffold
- Base cost on the culturing of cells and materials
  - Price will be influenced by market demand and competitor's prices
- Fees to be covered by health insurance by patient

Demand

- 17 million people reported have knee problems in 2000
- Total knee replacements cost insurance companies $41 billion annually
- Osteoarthritis leading chronic condition in elderly
- Cartilage repair has extremely limited treatments available

Competition

- Carticel®
  - Autologous cultured chondrocytes
  - First company to have FDA approval for cell therapeutics
  - Many insurance companies cover the treatment
    - Chondrocytes cultured and regenerated in defective site
  - Only major competitor
  - Average cost of their services - $10,360

Strength of N.K.O.B.®

- Non-invasive
  - Reduced surgery costs, recovery time
  - Fewer revisits to physicians
- Regeneration lasts longer than knee replacements
  - Advantageous for younger patients
- Treatment will allow repair to underlying bone
- Pricing model will account for both strengths and weakness in determining selling price

Current Costs

- Total knee replacement: $25,000
- Other arthroscopic surgeries: $5,000-$10,000
- Carticel® treatment: $26,000
  - Genzyme Tissue Repair fee: $10,360
  - First year Carticel® brought in $29 million in sales
  - 136 million people have insurance companies that cover Carticel® treatment

Selling Price

- Pricing Model
  - Based on production costs, investments, FDA and clinical trial costs
  - Uses superiority and inferiority functions
  - Evaluates current demand for treatment and approximates increase in demand
  - Uses competitor's prices as basis for estimation

Capital Investment

- Major Cost – construction of new facility
  - 2 major laboratories, 1 animal storage, 1 cellular storage, 3 offices
  - 10,000 square feet
  - Construction + furnishings = $3 million
- Other investment costs:
  - Equipment: $76, 300
- FCI: **$3 million**

Clinical Studies Cost

	Cost	Cost/5 yrs
1st Stage		
Labor	$630,000	$3,150,000
750 Patients	$50,000	$37,500,000
Hospital Fees ($10,000 per patient)	$10,000	$7,500,000
Utilities (Refrigeration)	$12,000	$60,000
Misc. Operating Costs	$5,000	$25,000
2nd Stage		
Labor	$950,000	$4,750,000
750 Patients	$50,000	$37,500,000
Hospital Fees ($10,000 per patient)	$10,000	$7,500,000
Utilities (Refrigeration)	$12,000	$60,000
Marketing	$10,000	$50,000
Misc. Operating Costs	$5,000	$25,000
	Total	$98,120,000

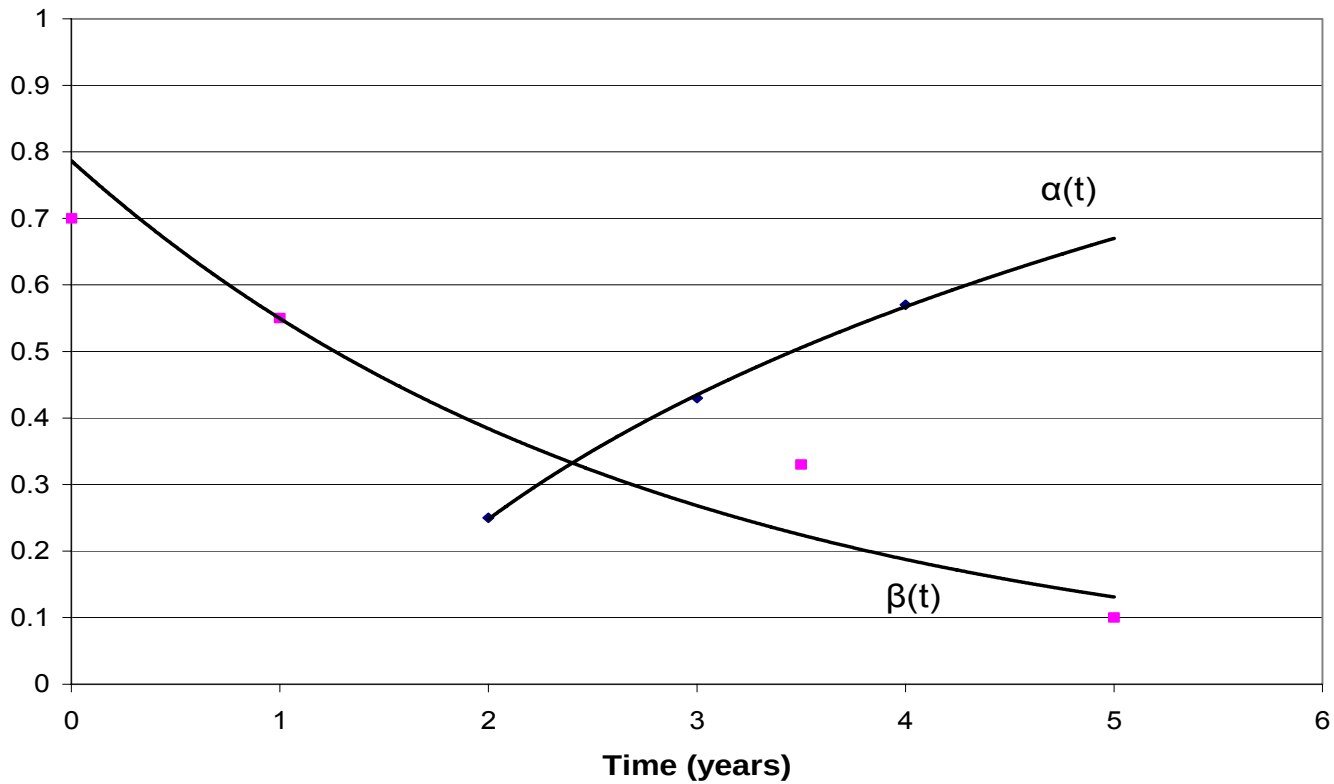
Production Costs

- Raw material cost per implant
  - Cell harvesting - $167.49
  - Polymer production and cross-linking - $5.36
  - Gelatin microcapsules and growth factor microsphere - $250.66
- Total production cost per implant
  - **$423.52**

Pricing Model

- Model uses demand and selling prices of competitors as well as superiority and inferiority functions

$$\beta(t) \cdot p_1 d_1 = p_2 (D - d_1) \cdot \alpha(t)$$



Pricing Model

- Pricing model manipulated to allow break-even point after three years

$$\sum_{i=1}^{3}\left[\frac{p_1 p_2 D\alpha_i}{\beta_i p_1 + \alpha_i p_2} - \frac{pc \cdot p_2 D\alpha_i}{\beta_i p_1 + \alpha_i p_2}\right] = FCI + FDA$$

  - *p_2=$10,360/implant*
  - *D=25,000 implants*
  - *pc=$423.50/implant*
  - *FCI = $3 million*
  - *FDA+clinical trials = $100 million*
- Selling price, p_1, of N.K.O.B.® services: **$11,000**
- Surgery cost will be comparable to arthroscopic surgery ($5,000 - $10,000)

Cash Flow

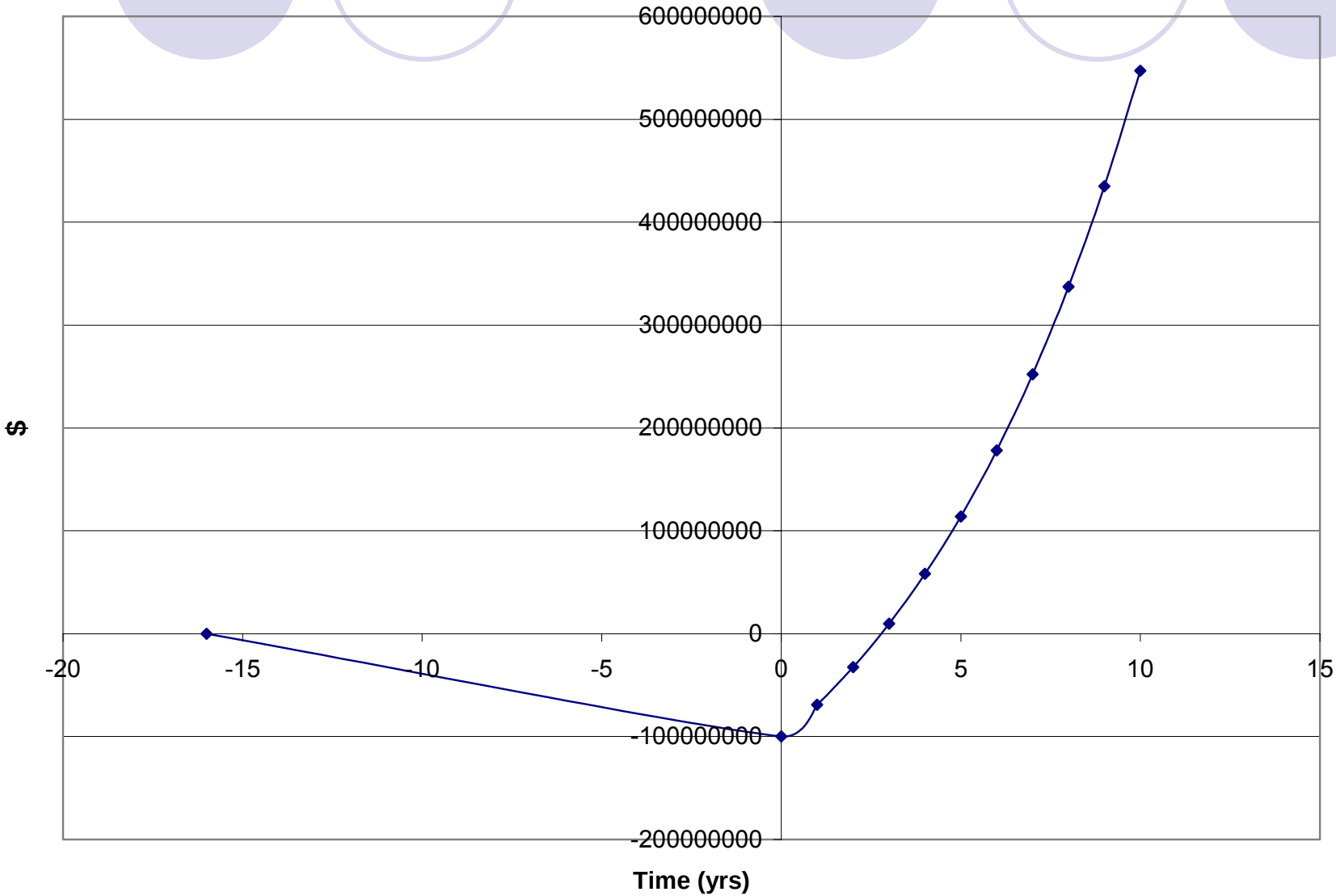
Year	Raw Material Cost (2% inflation rate)	# of implants	Revenue	Total Production Cost	Cash Flow
1	$423.50	3000	$33,000,000	$2,025,500.00	$30,974,500.00
2	$431.97	3450	$38,709,000	$2,137,296.50	$36,571,703.50
3	$440.61	3968	$44,515,350	$2,395,117.79	$42,120,232.21
4	$449.42	4563	$51,192,653	$2,697,542.17	$48,495,110.33
5	$458.41	5247	$58,871,550	$3,052,285.97	$55,819,264.41
6	$467.58	6034	$67,702,283	$3,468,400.44	$64,233,882.49
7	$476.93	6939	$77,857,625	$3,956,502.72	$73,901,122.65
8	$486.47	7980	$89,536,269	$4,529,046.69	$85,007,222.49
9	$496.20	9177	$102,966,710	$5,200,640.77	$97,766,068.79
10	$506.12	10554	$118,411,716	$5,988,420.62	$112,423,295.37

Raw material prices reflect 2% inflation rate

Cash Flow = Revenue - Total Production Cost

Demand for implants modeled after Carticel® demand

Cumulative Cash Position



Summary

- Goals
  - Provide non-invasive solution to cartilage and bone repair at competitive prices
  - FDA Approval
  - Profit
- Advantages
  - Comparably non-invasive and affordable
- Selling Price
  - $11,000 plus approximately $5,000 in surgery fees

Thanks to…

- Dr. Bagajewicz
- Dr. Sikavitsas
- Kim Fink
- Chem-E class

Any Questions?