RepliDerm Inc.

AN ADVANCED DESIGN PROJECT

Submitted to Dr. Bagajewicz

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GROUP 8

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INTRODUCTION

Mission Objective

RepliDerm Inc. was assigned the task of creating a new allograft for the care and treatment of burns. Our original mission was to create a skin replacement using human donor skin taken from cadavers that had been decellularized. It was soon discovered that the use of donor skin to create a skin tissue implant would produce an inferior product in comparison to skin tissue implants derived from alternate sources. The human skin was difficult to decellularize to prevent immune rejection[1] and did not heal as quickly and cleanly as other implants, and often had tissue rejection problems. After speaking with health professionals at the Baptist Integris Burn Center in Oklahoma City and listening to their dissatisfaction with and the reasons they refused to use human-sourced allografts, it was soon decided that using decellularized skin was not the best choice to create a skin tissue allograft. It was also discovered that there were other markets besides burn injuries (skin tissue damage from diabetic ulcers and from necrotizing infections) that were more in need of skin tissue implants.

Background

In the United States, there are 51,000 burn victims annually who will receive a graft of some size to heal their wound. Since the mid-1980's, the number of burn victims in the U.S. has decreased due to better work place safety and burn prevention education. It is for these reasons that most hospitals and major burn centers have begun to diversify and treat other ailments such as diabetic ulcers and large tissue wounds.

There are currently 1.5 million diabetic patients in the U.S. annually who develop a wound (generally on the lower extremities) related to their diabetic condition that seek treatment requiring a graft or graft-like implant. This number is expected to grow as high as 14 million patients annually over the next ten years as diabetes becomes one of the largest health concerns in an aging U.S. population. This rapid expected increase of wounds that will need treatment has garnered the attention of many major burn centers, now most of which treat wounds as well as burns. The treatments of diabetic wounds, which usually appear in the form of ulcers on the feet, are similar to skin wounds received from injury. However, wounds in patients with diabetes generally are much more prone to infection because of their slowness in healing and their location on the patient's feet. Although the diabetic wound market is much larger than the burn wound market, it should be noted that burn victims typically would need much more skin tissue implants due to the nature and size of the typical injury.

In 1980, Yannas and Burke developed a bilayered dermal regeneration template using primarily refined collagen from bovine tendons cross-linked with chondroiten 6-sulfate from shark cartilage[6]. Chemically, this material resembles the acellular component of the dermal portion of skin with which it shares many properties such as tensile strength and flexibility. On top of the material is a thin coat of silicon rubber that protects the implant from cold, heat, and foreign antigens while allowing the flux of water at a rate similar to that of actual human skin.

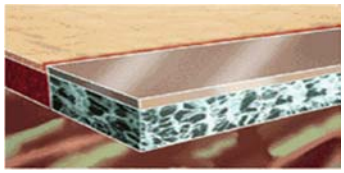
When this product is placed on a wound, the body will begin to vascularize the collagen matrix. As blood vessels form, the shear force on the matrix breaks it down into its small constituent components, which are flushed and degraded in the body. The body

replaces the artificial, randomly organized collagen matrix with its own highly structured matrix. The body's own healing process, when supplemented with the tissue graft, regenerates the dermal layer of skin with less scaring and in a shorter time. When the implant has been entirely vascularized and the majority of the artificial collagenous material replaced, the top silicon layer is replaced with a meshed epidermal autograft from the patient.

According to published multicenter clinical tests, the median take of such a graft is 95%, which is comparable to the take of autografts[2]. Dermal regeneration templates are of great benefit because when used, they heal with much less scarring at the patient's donor sites.

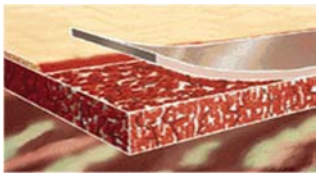
Our Solution for the Demonstrated Need

The average patient's body will vascularize Yannas' and Burke's collagen matrix in 21 days. During this time, the patient is especially susceptible to infection. Since each day in the hospital results in an average cost of $10,000 to the patient, a shorter vascularization time greatly decreases the treatment cost and time for the patient.
The diagram[3] on the following page gives a pictorial representation of RepliDerm and the body's response to treatment. RepliDerm improves the design of Yannas and Burke by releasing an angiogenesis-stimulating growth factor that effectively shortens the vascularization healing time.



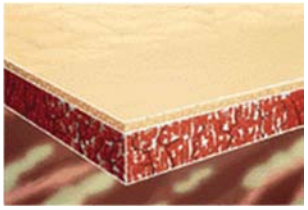
1. RepliDerm placed on Damaged A
(Day1)

2. Vascularization in progress
Matrix.
(Day 7-14)



3. Silicone Removed
(Day 14)

4. Meshed Epidermal
Autograft added
(Day 14)



5. Skin fully healed
(Day 21)

Figure 1: Healing process of Skin Template Replacement

The growth factor VEGF (vascular endothelial growth factor) stimulates angiogenesis that increases the speed and neatness in which the body heals, and thereby decreases the time required for a patient's recovery. In biological systems, VEGF has a very short half-life (1-2 days)[4]. Benefits of treatment with this growth factor would be very small (3-5 days at maximum) if there were no means of releasing VEGF in a controlled manner. By incorporating VEGF into microparticles made from a slowly-biodegrading polymer such as poly-DL-lactic-co-glycolic acid (PLGA), the release of VEGF can be regulated to a consistent and lasting release rate. As the PLGA polymer degrades, the growth factor incorporated in the polymer is released. Different microparticle sizes, the location of the microparticles, and shear stresses on the microparticles will affect the rate at which the polymer degrades. The PLGA

microparticles are incorporated into the collagen matrix of RepliDerm to prolong the benefits of increased angiogenesis throughout the entire recovery time of the patient.

Synthetic grafts generally heal more quickly and with less scaring in comparison to the cell-based grafts. Synthetic grafts also have longer shelf lives relative to the cell based grafts and are also less prone to rejection by patients. Synthetic grafts cost more than the cell-based grafts; however, given the extra expense involved in the incineration of human cell-based waste, the cost of synthetic grafts is worthwhile. We have determined that the increased cost of producing synthetic grafts is justified by the fact that they heal wounds with much less scarring and in shorter time compared to cell-based grafts.

Currently, there are several companies that are using synthetic skin grafts to treat burns and wounds. However, none of these companies incorporate any form of a growth factor release system into their grafts. We propose to incorporate a time-released vascularization growth factor into the synthetic graft to shorten the healing time and to improve the appearance of wounds that can be treated using RepliDerm.

RepliDerm Production Process

Objective

RepliDerm is an artificial skin template that can be used as a skin replacement for damaged skin. In addition, RepliDerm has an advantage over other products because it contains growth factors encapsulated in degradable microbeads that stimulate the body's natural healing process. As the microparticles break down, they release an angiogenesis-stimulating drug into the surrounding artificial tissue, which speeds the vascularization and healing process of the wound. The controlled release system makes RepliDerm a superior product in comparison to other skin replacement products currently on the market.

VEGF Controlled Released Microsphere Production:

The method of production described here is based on the solid encapsulation/single emulsion/solvent extraction techniques of King and Patrick[4]. Advantages to this technique for solid encapsulation of proteins (over the technique of Cao and Shoichet[5] for example) include relative inexpensiveness of operating equipment, versatility of production quantity, simplicity of scaling and fewer required sterilization procedures[4]. Tables 1 and 2 show the materials and equipment required along with their respective costs.

Table 1: Raw material needed for microbead production

Needed Materials:	Source:	Amt/sheet	Cost of Materials:	Cost /sheet
PLGA	Birmingham Polymers	387mg	$8.60/g	$3.30
PEG	Sigma-Aldrich	3.87mg	$323.20/g	$1.25
Methylene Chloride	Dow Corning	3mL	$10.00/L	$0.03
VEGF	Peprotech	38.7µg	$3600/mg	$139.32
Human Serum Albumin	Sigma-Aldrich	77.4mg	$4.56/g	$0.35
PVA	DuPont	100mL	$0.17/L	$0.02
Isopropyl-alcohol	Acros	100mL	$0.34/L	$0.03
Miscellaneous laboratory supplies: latex gloves, Pasteur pipettes, paper towels, autoclave trays and bags, etc.				
Total Materials Cost:				$145.00

Table 2: Equipments needed for microsphere production

Needed Equipment:	Source:	Cost of Equipment:
10 100mL Pyrex bottles		$400
10 1000mL Pyrex bottles		
5 small		$50
5 large magnetic stir bars		
5 Bellco Carrier Magnetic Stirrer	American Laboratory Trading (ALT)	5@$325
1 Magnetic retrieval rod		$25
2 Mettler Balance Model AE200	ALT	2@$2,000
1 OPS Vortexer Model TR-945007	OPS Diagnostic	$1,600
1 Large centrifuge Beckman XL-90	Beckman	$16,900
1 VWR Vacuum Oven Model 1410	VWR Scientific	$895
1 Forma Scientific Freezer Model 786	ALT	$3,500
1 Wet/Dry AMSCO Cyclomatic Autoclave	AMSCO	$5,000
1 Nuaire Biosafety Cabinet NU430-400 Class II Series 24	ALT	$3,900
Total Equipment Cost		**$37,245**

The majority of the production process will take place in the laminar flow hood. The hood must be sterilized periodically with a topical agent (70% EtOH) every half hour or so when in use. When the hood is not in use, a UV lamp should be turned on to kill any microbes that possibly survived topical sterilization.

A flow diagram, figure 1, shows the production of the PLGA microspheres containing VEGF. A description of the process follows the flowchart on the following page.

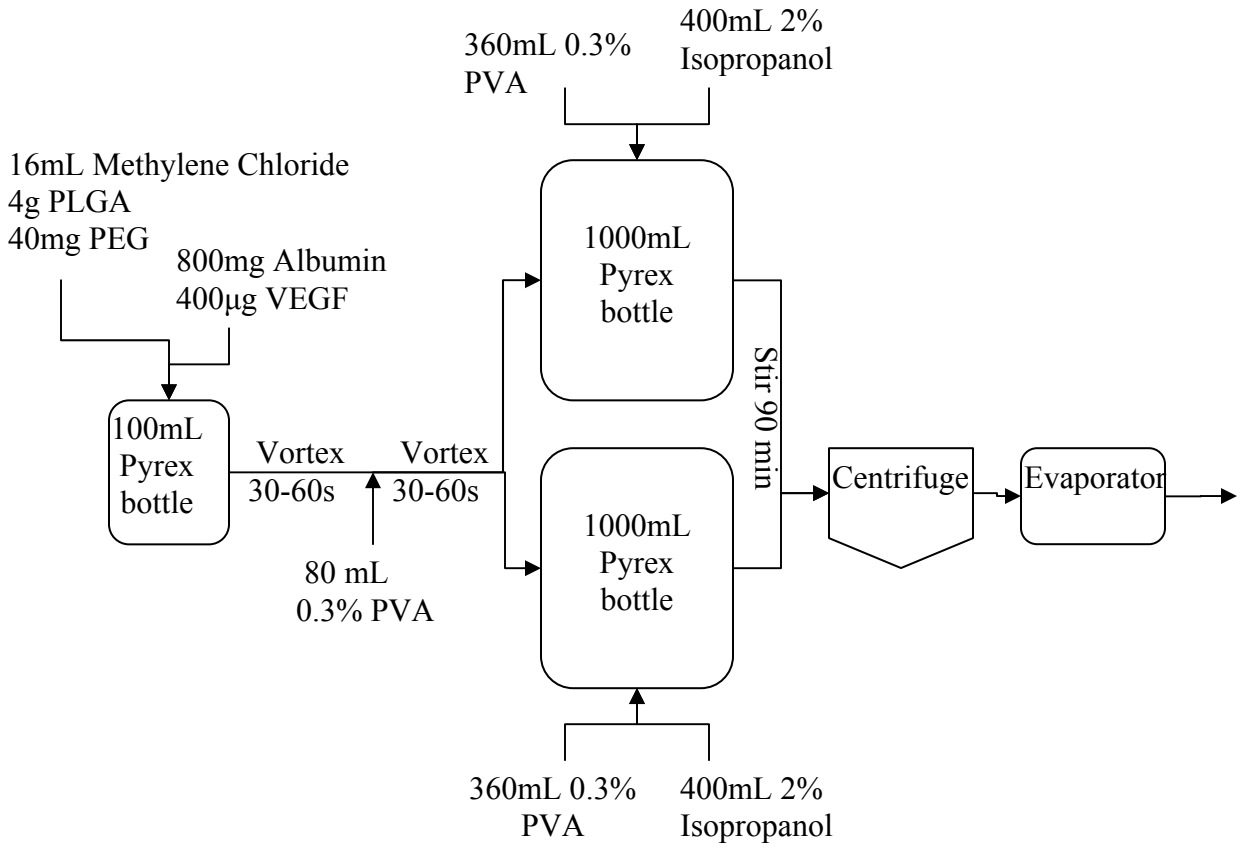


Figure 2: Flow chart for the production of the microparticles

Description of Microbead production process

The following steps describe the method to produce the microbeads used in RepliDerm:

1. In the laminar flow hood, mL methylene chloride and one small magnetic stir bar is added to one 100mL Pyrex bottle (previously sterilized in the autoclave). This bottle is placed on the heated stirring platform (still inside the hood) with the stirrer set at a moderate pace. 4.0g of 50/50 poly (DL-lactic-co-glycolic) acid (PLGA) is added slowly to the stirring methylene chloride (PLGA is sold in small pellets that will clump together in the solvent if added all at once). By gradually adding the pellets while the solvent is stirring, the pellets remain mostly separated and dissolve much more quickly.
2. After the PLGA has been added and is dissolving, 40mg of polyethylene glycol (PEG) is added to the stirred solution. These proportions of PLGA and PEG produce microcapsules that dissolve entirely in vivo within four weeks. The proportions mentioned are those used by King and Patrick, but the amount of PEG can be increased to tailor the degradation rates to the optimal rate determined through experimentation. While no literature has been found correlating the degradation rate of the polymer to the proportion of PEG, it is clearly not a linear relationship but may be determined through experimentation.
3. Prior to the addition of VEGF to the solution, 800mg of albumin is added as a stabilizing agent while the solution continues to stir. Without a stabilizing method of some sort, methylene chloride (or any known possible organic solvent) causes the unfolding and denaturing of VEGF. Albumin acts as a kind of buffer

protein, insulating the VEGF molecules and thus maintaining the integrity of its quaternary structure.

4. Following the addition of the albumin, 400μg of VEGF can be loaded with the assurance of minimal (if any) loss of bioactivity. Again, this is done while stirring.
5. With all the needed materials now in solution, the bottle is capped and the whole solution is vortexed vigorously for thirty to sixty seconds. The proteins are soluble both in water and in the organic phase; vortexing causes the extraction of the proteins from the aqueous phase in which it is added to the organic phase containing the polymers. It should be noted that the vortexer could be located inside the hood to prevent the possible introduction of bacteria and fungi to the sterile environment. If logistical problems prevent the vortexer from being located in the hood, the bottle must be sprayed with 70% EtOH immediately prior to being reintroduced into the sterile environment.
6. After vortexing, only one phase will exist (the organic phase). The bottle is reopened and 80mL 0.3% wt. polyvinyl alcohol (PVA, prealiquoted and sterilized) are added in the autoclave wet cycle. The bottle is resealed and vortexed on vigorously for thirty to sixty seconds. At this point, the methylene chloride is extracted into the alcohol phase and the polymer containing the albumin and VEGF precipitates out of solution. At this stage of the process, the size of the beads can be controlled by varying two parameters.
7. The contents of this 100mL bottle are now transferred to 2 sterile, autoclaved 1000mL Pyrex bottles with large magnetic stir bars (48mL solution to each bottle

respectively) in the flow hood. 360mL 0.3%wt (PVA) and 400mL 2% Isopropyl alcohol are added to each of the larger bottles. The large bottles are placed on the stirring platform with the stirrer on medium to medium high speed for 90 minutes. This step extracts any residual methylene chloride in the polymer/protein particles.

8. The microspheres are now completely formed but require separation from solution. This separation is performed in two stages. First, the solution is transferred to sterile, autoclaved polystyrene 250mL centrifuge tubes which are then capped and removed from the hood. In a large centrifuge, the solution is spun at 7000 rpm for 10 minutes. The supernatant is discarded in a biohazard waste container. The exterior surface of the 250 mL tubes is sprayed with alcohol and reintroduced to the hood.
9. While still inside the hood, the containers are uncapped and placed inside a vacuum dryer to evaporate the residual water and alcohol.

Once dry, the microparticles, resembling a powder or fine table salt, can be collected with a sterile metal spatula and stored in a sterile polystyrene falcon tube in a freezer at -20°C.

Review of production process

The entire process for producing RepliDerm will be reviewed in the following section. In order to understand the process and requirements of the entire production, the following points will be discussed:

- Raw material needed
- Equipments needed
- Flow Diagram of Process
- Human labor needed
- Facility lay out

Raw Materials

The following table summarizes the amounts of raw materials required for entire process of RepliDerm production for 6”x 4” sheet and their respective costs. From the production and raw material costs, the total production cost per sheet is calculated as shown on the following page.

Table 3: Raw materials needed for RepliDerm production

Process Agents					
Raw materials	**Use**	**Where to obtain**	**Cost**	**Amt/sheet**	**Cost / sheet**
Bovine tendon collagen dispersion Cow Tendons	Used to make matrix	Eastern Regional Research Center, U.S.D.A. in Philadelphia	$165 per mL	0.310mL	$51.15
Chondroitin6-sulfate solution (Shark Cartilage)	Used to make matrix	Sigma-Aldrich Chemical in St. Louis.	$18.32 per gram	0.0155g	$0.28
Silastic [poly (dimethyl siloxane) prepolymer]	Used as a temporary barrier to protect against infection, shear force in the new tissue and loss of electrolytes	Dow Corning.	$1 per square foot	0.167 ft^2	$0.17
Glutaraldehyde in 0.05 M acetic acid	To obtain saline medium	Dow Corning.	$66.50/gal		
Microbeads Capsules with VEGF inserts	Time-released growth factor into the synthetic graft to shorten the healing time	See Microbead production process above			
Total Cost/sheet			**$195.50**		

The thickness of the collagen portion RepliDerm dermal regeneration template is one millimeter, the thickness of the natural dermal portion of skin. The finished product has a pore volume fraction of 98%. The actual material required for the production, therefore is 2% of 15.5cc or 0.3097cc, 95% of which is collagen. The remaining 5% is chondroiten 6-sulfate. Accordingly, $0.95 \cdot 0.310cc = 0.294cc$ of collagen is required per sheet. The density of the dermal matrix is almost equal to that of water, about 1.0g/cc, so $0.05 \cdot 0.310cc \cdot 1.0g/cc = 0.015g$ of chondroiten -6-sulfate is required per sheet.

According to King and Patrick[4], 125mg of microbeads containing 0.01% VEGF by mass per 5mL BSA solution provided sustained release of VEGF to maintain 5-10ng/mL concentrations in conditions mimicking human tissue. According to reports by

Mooney[6], this concentration is optimal to encourage new vessel formation. The PLGA required for production of one sheet is therefore:

$$\frac{125mg}{5mL} \cdot 15.5mL = 387mg / sheet$$

The microspheres are about 1% PEG by mass. Therefore, 3.87mg/sheet PEG are required for production. The VEGF required for one sheet is therefore:

$$0.387g \cdot 0.0001 = 38.7\mu g / sheet$$

Albumin is added at a ratio of 2,000 to one with respect to VEGF. Therefore:

$$38.7\mu g \cdot 2000 = 77.4mg / sheet$$

Equipment Needed

The equipment that is required in the RepliDerm production process is listed in table 4 below:

Table 4: Equipment needed for RepliDerm production

Equipment	Use	Where to obtain	Unit cost of equipment
20 Stainless steel pans	To allow matrix gel to set	Fisher Scientific	$49.00
3 Freezers With Temp range -50° to -86°C	To aid formation of microspores	Fisher Scientific	$8,381.39
2 Vacuum Oven2	To increase the strength of crosslinkages	Fisher Scientific	$2,984.22
Blender/ Homogenizer	To mix the collagen cartilage and acidic solution for matrix formation	Fisher Scientific	$8,582.00
Mechanical Spreader	For Silastic Application	Fisher Scientific	$3,341.87
10 Plate Shaker	To for uniform spread of microbeads	Fisher Scientific	$1,149.00
Room Sterilizer	Kills surface bacteria	Fisher Scientific	$8,265.87
Sterile Hood	Processes after the vacuum will take place under the hood		$6,273.00
Distiller	To obtain distilled water for elution		7,233.00
Autoclave	For sterilizing smaller instruments	Fisher Scientific	$3,970.25
Microcapsule Equipments	See Microsphere production above		$37,245
Total Equipment Cost			**$108,152.6**

RepliDerm Production Process

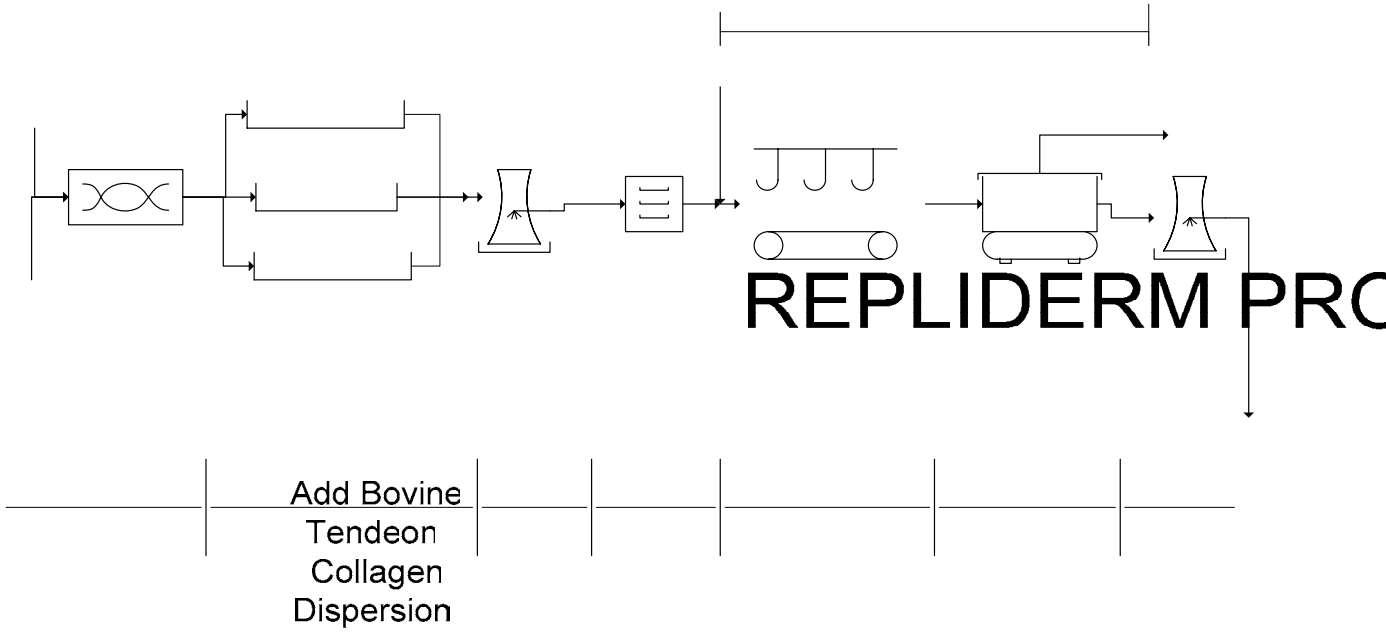


Figure 3: Diagram of the RepliDerm production process

Description of the process

As mentioned earlier, RepliDerm is a synthetic decellularized skin template. Synthetic grafts generally heal quicker and with less scaring than the cell-based grafts. In addition, RepliDerm has an advantage over other processes because it contains microbeads with a time-released growth factor into the synthetic graft to shorten the healing time. The production process is quite straightforward but requires a highly sterile environment. The production process is discussed below:

1. First, collagen from bovine tendons is crosslinked with chondroiten 6-sulfate and glutaraldehyde to form a tough and supple material similar to actual skin[1]. This is

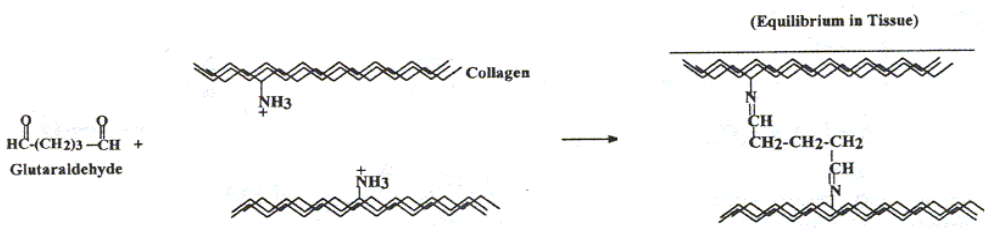
done by using the homogenizer equipment. Chondroiten6-sulfate is added in a drop-wise to the collagen dispersion in a homogenizer.

2. The precipitate is filtered and cast in stainless steel pans to set.

3. Once set, the gelatinous material is freeze-dried in a -60ºC freezer. Freezing results in the formation of pores throughout the material; the final porosity is 98% with the mean pore diameter being 150μm[1]. The porous material at this point of production is weakly crosslinked and will revert to gelatin in the presence of water[7].
4. The material is placed into a vacuum oven at 105ºC and 50 mTorr to promote internal crosslinking and to increase mechanical strength.
5. The next step is the addition of the microbeads into the matrix. In order for the microbeads to be incorporated, they are sprinkled over the top of the sheet. After implantation, the diffusion of the VEGF will create a concentration gradient with the highest concentration near the top of the implant as the VEGF in the lower portions of the implant is carried away by vascularization.
6. Following the addition of the microspheres, a thin (0.1mm) layer of silicon rubber is laid on top of the collagen matrix. This is done with a mechanical spreader to

allow uniformity. It is important that this step be done following the dehydration crosslinking when the matrix adheres most readily to the silicon[8].

7. The sheet will be placed in a bath solution of 0.25 % glutaraldehyde in 0.05 M acetic acid on top of a plate shaker at medium speed. This step increases the crosslinking density of the sheet and adds to the mechanical strength of the material. The glutaraldehyde crosslinking process is a very critical step in the production of the dermal regeneration template. The crosslinking binds collagen strands to other collagen strands and collagen to the GAG through a dehydration reaction. This reaction takes place in an acidic medium in order to protonate the amine groups on the collagen and GAG. The aldehyde groups on the glutaraldehyde react with the protonated amine groups to form crosslinks and water[1]. The concentration of the glutaraldehyde in this step greatly affects the mechanical and physical properties of the collagen matrix. A higher concentration of glutaraldehyde means a higher degree of crosslinking or a smaller molecular weight between crosslinkages (lower M_c). This property, M_c directly corresponds to a series of physical properties of a crosslinked material, such as pore diameter and tensile strength. A concentration of 0.25% wt. corresponds to a M_c of 13,000 and a pore diameter of 250μm, ideal for vascularization of the matrix.



8. The next step is an elution of the formed aldehyde with distilled water.
9. The last step in the process is a final sterilization of the dermal replacement. The dermal replacement is irradiated and then freeze-dried. It should be noted that the addition of VEGF requires that the dermal replacement should be stored in either a refrigerator or a freezer. In a 4°C refrigerator, the sheets can keep for up to 2 months. In a -80°C freezer, the sheets can keep up to 2 years. Upon use, the material should be re-hydrated in a sterile saline solution for at least 30 minutes but no more than 4 hours.
10. The product is then packed up to 50 lbs in dry ice.

Upper Capacity Limits for the Described Production Processes

Two limiting aspects of operation must be considered in determining the production capacity limits to the production of RepliDerm templates. First, the production step that requires the longest amount of time must be identified. In any period of sustained constant production, this step will determine the shortest amount of time for which one unit can be produced. The other limiting quality to production is the equipment processing capacity (how much product can be made at one time).

For the production of the microcapsules, we are only limited by equipment capacity in the number of magnetic stirrers and of Pyrex bottles we possess. Other equipment, while having low capacities, are occupied for very short times for each cycle and therefore can be considered as having a much higher capacity.

For the microcapsule production process, we are only limited by equipment capacity in the number of magnetic stirrers and of Pyrex bottles we possess. Other

equipment, while having low capacities, are occupied for very short times for each cycle and therefore can be considered as having a much higher capacity.

The method previously described produces 4.0g of microcapsules per cycle. Up to three times the quantity designated by the production description can be run simultaneously and 90 minutes on average are required to complete each cycle. This means that in each 90 minutes of processing time, 12g of microspheres can be produced. In a ten-hour workday, this equates to 90g of microparticles that can be produced. Each sheet requires 0.387g, which means that one day of work can produce enough microparticles for 230 sheets. Working 26 days a month, this production capacity resolves to enough microparticles for the initial production of 6,000 sheets per month.

For the production of the matrix, our equipment capacity limitation is the number of stainless steel casting pans available. Each pan is one square foot; the dimension of one sheet of product is 24 square inches. Thus, each pan holds six sheets.

The time restricting step in the production of the matrix is the glutaraldehyde crosslinking that takes two hours. If ten pans are in operation at the glutaraldehyde crosslinking stage of production at any given time, the production capacity is 60 sheets every two hours or 30 sheets an hour. This corresponds to a total capacity of 18,700 sheets per month, far exceeding the production capacity of the microparticle production.

Equipment as described is sufficient for initial production goals of 2200 sheets per month. Increasing production capacity is relatively inexpensive since the equipment with the limiting capacity in production of both the microspheres and matrix is very inexpensive. Increasing production capacity can be done simply by purchasing more Pyrex bottles, more magnetic stirrers, more stainless steel pans and more mechanical

shakers. The cost of these items is almost insignificant when compared to the cost of such equipment as the centrifuge and the autoclave.

Required Human Labor

The minimum numbers of employees we will need at any point in time is one Ph.D., 3 lab technicians, and 2 administrative assistants. This number will be required at the initial start up of the company for the pre FDA tests. When our company grows and enters the FDA clinical trail, we will increase the size of our staff to accommodate for the increase amount of work as needed (advertising, production, etc.). Finally, we estimate the amount of our total staff and personnel to be more than 50 for the first year our company enters the market.

Table 5: Cost of Maintaining Personnel

		FDA			Market	
	Laboratory Testing	Phase 1	Phase 2	Phase 3	Year 1	Year 2
No. of personnel	6	10	20	30	60	100
Cost	$360,000	$600,000	$1,200,000	$1,800,000	$3,600,000	$6,000,000

Facility Specifications

The minimum requirements for a RepliDerm production facility are given in the following list:

- Offices for 2 Administrators, 1 Ph.D., and 3 technical assistants
- Cryogenic room

- Reproduction rooms
- Product Storage room
- Animal Storage
- Lab Testing Facility

It is estimated that roughly 30,000 sq-ft of floor space will be required for RepliDerm's production facility. The facility will contain both the laboratory and business sectors. The business facility will consist of small office spaces for each employee and with a larger conference room for meetings. The rest of the facility will be laboratory space, with roughly 3,000 square feet set aside for animal storage. The laboratory space will have a large amount of bench space for working with experiments and samples. In addition, two "clean rooms" for creating the product and one cryogenic room (containing -80°C freezers) for processing and storage the product will be needed in the facility. The building price of the laboratory includes furnishing the laboratory with standard laboratory equipment (fume hoods, gas lines, and everything else that is "built into" a laboratory). The price of our facility is based off of the average price per square foot obtained from several other different biomedical research facilities (H. Lee Moffitt Cancer Center & Research Institute - Cancer Research Center, Johns Hopkins Oncology Laboratory - Bunting Blaustein Research Building , MD Anderson Cancer Center - Basic Sciences Research Building, National Institutes of Health - Mark O. Hatfield Clinical Research Center, University of California, San Diego - Rebecca and John Moores Cancer Research Center, and the cost per square foot of furnished laboratory facilities was determined. The average cost per square foot was just over $275 (with an average of ten

percent of the space for animal facilities), meaning a laboratory of 30,000 square feet would be estimated to cost $8.25 million dollars.

FDA Approval

Approval from the Food and Drug Administration (FDA) is the most crucial step of starting RepliDerm Inc. because it takes a large amount of time and money. As a result, it is critical to take into account all of the possible outcomes of the procedure. Since RepliDerm is a medical device, it will go through very rigorous FDA approval procedure.

The branch of FDA that grants approval to medical devices is the Center of Devices and Radiological Health (CDRH). Under the CDRH, devices are further classified into class I, II and III devices. RepliDerm falls under the class III devices. According to FDA definitions, Class III devices are devices that support or sustain human life, are of substantial importance in preventing impairment of human health, or present a potential and unreasonable risk of illness or injury. The FDA requires that all class III devices go though a process known as the Pre-market Approval process (PMA). The PMA is a scientific and regulatory review to evaluate the safety and effectiveness of the device; it is the first step in the process that will eventually lead to FDA approval. The following diagram shows the general flow of the process for FDA approval.

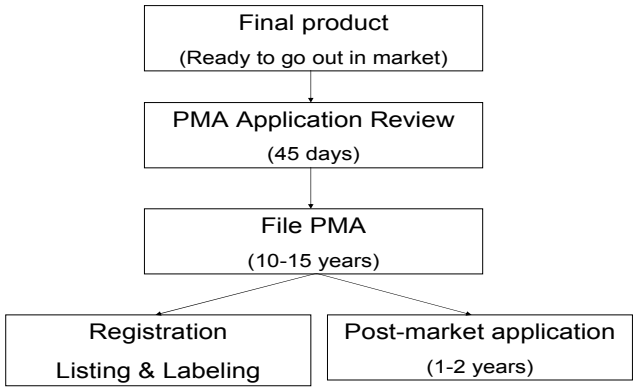


Figure 4: FDA Approval Procedure

PMA Application Review

Two years of laboratory experiments will be conducted to develop RepliDerm; this amount of laboratory testing is a mean value used by other companies (most notably the product Integra) creating similar dermal replacements to prove its safety and efficacy for the FDA Pre-Market Approval process. The FDA Pre-Market Approval Application process takes between 45 to 60 days to review. Once the Pre-Market Approval Application is complete, then full testing can begin. The following issues can be identified as possible reason for refusal of filing PMA application:

- Lack of general information/requirements (such as missing signatures or information)
- Information placed in the Table of Content of the PMA application
- Vagueness in the directions for use of the device
- Alternative practices and procedures for the product mentioned but not outlined in the procedure

Any of the above points may be used as grounds to fail an initial PMA application. Including delays, the PMA application review process can take between six and twelve months. The cost of the PMA process is between $50,000 and $60,000.

PMA Application Procedure

The standard fee established by the FDA for the PMA application is $206,811 (1). This procedure is stringent and complicated because it involves clinical trials and pre-approval investigation of manufacturing facility. There are two ways to file for PMA application: by Modular PMA (1) or Traditional PMA (2). Even though RepliDerm Inc. has a choice between the application procedures mentioned previously, it is more beneficial for RepliDerm Inc. to use Modular PMA. The table on the following page shows the advantages and disadvantages for both of the procedures.

Table 6: Advantages and Disadvantages of Modular and Traditional PMA

Modular PMA	Traditional PMA
Advantages	**Disadvantages**
Faster because after submitting one module, FDA starts reviewing the application and a company would start working for next module.	Time required collecting the data plus time for them to review everything submitted.
There is potential to expedite PMA review process because of industry support.	As few as 10% of new applicants pass FDA review on initial trial.
Cost less to go just one step back and modify or restart the required procedure	FDA suggestions and revisions can result in significantly higher costs that must be borne by the manufacturer.
Takes less time because when something is disapproved only one step needs to go back.	If something is disapproved in early studies, data used from that step for the rest of the studies are useless and reproduce all the results.
Disadvantages	**Advantages**
It can get caught up in bureaucratic "red tape". FDA review panel because things are done simultaneously	Potentially less "red tape".
Companies need to respond promptly to FDA questions for each	There are no major deadlines for responding to FDA review questions. There is only one or two deadline to worry about for entire PMA approval process
Not acceptable for devices previously approved in foreign countries with established health administrations.	It is recommended if the clinical studies have been approved in a country with established health administration.

As it is shown in above table, the advantages of the Traditional PMA process are not as significant and can be achieved by a Modular PMA process. The disadvantages associated with the modular process can be overcome if frequent and open communication is maintained with the Office of Device Evaluation (ODE).

For the aforementioned reasons, RepliDerm Inc. has chosen to file a Modular PMA application. The Modular PMA submission is divided into the four different modules listed below:

1. Module 1 Non-clinical laboratory studies phase 1 (cellular) and phase 2 (animal) testing
2. Module 2 Manufacturing Information module
3. Module 3 Final PMA (human clinical studies) module

Pre-FDA Laboratory Testing

Prior to taking RepliDerm Inc. to the FDA for evaluation, several decisions must be made. First, RepliDerm Inc. must decide on the number of personnel to hire. RepliDerm Inc. has evaluated the advantages and disadvantages of three different workforce sizes: 1 Ph.D. with 3 lab technicians, 1 Ph.D. with 5 lab technicians, and 1 Ph.D. with 7 lab technicians. The reason the RepliDerm Inc. has chosen to go with only one Ph.D. is cost. The estimated salary for a Ph.D. in the biomedical engineering and sciences field is approximately $100,000. This salary is a bit prohibitive, and makes it difficult to hire more than one Ph.D.-level employee. The hiring of more lab technicians allows for more tests to be run concurrently, and for RepliDerm Inc. to save time. With more lab technicians, RepliDerm Inc. will be able to bring a product to the FDA quicker, run more tests in order to have a better chance of passing the FDA on the first try, and be able to start selling products on the market quicker.

The second major decision that RepliDerm Inc. has to make is to choose the number of experiments to run prior to bringing a product to the FDA for evaluation. The

more testing that RepliDerm Inc. can perform on their own, prior to FDA evaluation, the greater likelihood that RepliDerm Inc. will be able to pass the FDA trials on their first attempt. Of course, RepliDerm Inc. must balance the number of experiments run with the amount of time and money they are willing to spend. Fewer experiments will mean that RepliDerm Inc. will be able to begin FDA trials quicker. Of course, fewer experiments also mean that the chance of passing the FDA trials on the first try is significantly reduced.

All of the decisions that RepliDerm Inc. must make prior to taking a product to the FDA evaluation are first stage variables, or decisions. A first stage variable is a decision that must be made prior to an action being taken. For RepliDerm Inc., deciding factor in this decision is the amount of initial funding that they have. The initial funding will come from government granting agencies, such as the National Institute of Health, National Science Foundation, and the Center for Disease Control. RepliDerm Inc. will need at least $345,000 to begin applying for the FDA approval process.

A decision tree that illustrates the first stage decisions that RepliDerm Inc. must make is shown on the following page. The number of personnel affects the amount of time that RepliDerm Inc. will spend conducting tests. The number of experiments, or experiment set, chosen will affect the probability of passing the FDA trials and of certain failures occurring.

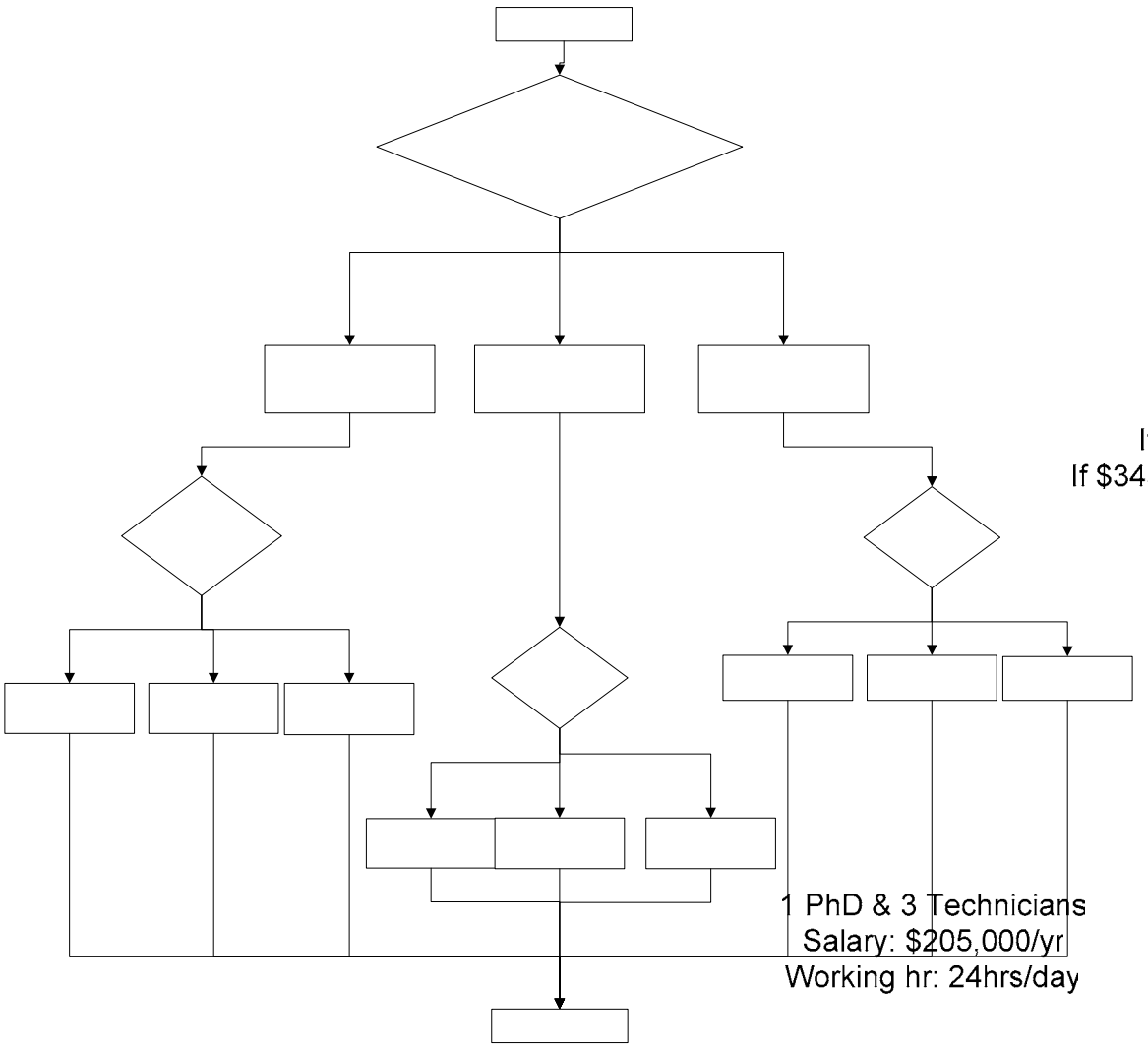


Figure 5: First Stage Decisions

In an effort to improve the chances of passing the FDA approval process, several tests will be conducted prior to submission to the PMA modular testing. A list of experiment sets that will be done by RepliDerm Inc. is on the following page. All of the experiments will serve to simulate the same types of tests that the FDA will use to evaluate RepliDerm Inc., their product, and their production procedure.

Experiment Sets

The following is a list and description of the cellular level tests to be run prior to FDA evaluation:

Cellular Level Flask Tests

1. Take a small vial of Human Vascular Endothelial cells that are pre-prepared, 2-3 mL, dermal cells from Sigma-Aldrich and transfer to a larger culture flask.
2. Grow the cells in the culture flask until they reach a confluency of 15%. The cells should be added to a petri dish that is 1 mL in volume. Ten milliliters of DMEM growth media should be added to the flasks, and the flasks then placed into a three gas incubator set to 37 degrees C for a period one day. This should take roughly one day; however, cell growth may vary by a day or two depending on conditions within the lab.
3. Cut an already formed dermal sheet into a 2 inch by 2 inch square.
4. Place the dermal sheet gently onto the top of the dish.
5. Gently push the dermal sheets onto the cells until the cells are in direct contact with the dermal sheet.
6. Let the sheets sit on the cells for a period of 3-4 weeks. Check the cells every 6-8 hours during the 3-4 week period to see if the sheets are intact and the cells are still adhered to the sheets. Change the media every two to three days so that the cells continue to grow and to remove cellular waste. When changing the growth media, be sure not to disturb the cells.
7. Note any changes or abnormalities. Be sure to observe at what point the dermal sheets degrade and are no longer whole.

8. Examine the cells and the dermal sheets for any apoptosis and cell growth.
9. Run a Caspase 3/7 assay to determine the amount of cell death in the flasks. If the amount of cell death after 4 weeks is greater than 50% (50% would be the normal amount attributed to natural cell growth), then the dermal sheets are most likely the cause of the cell death. If the amount of apoptosis is higher than normal (greater than 35%), then the dermal sheets are responsible for the controlled suicide of the cells. This means that the dermal sheets are toxic and should be re-evaluated.

NOTE: All of the experiments above are for one person. One person running cell-flask tests can, assuming an eight hour work day, can manage 50 flasks. This means that one person can run 50 experiments per week. Multiple tests can be run concurrently.

Chorioallantoic Membrane (CAM) Tests

1. Take fertilized leghorn chicken eggs that have been fertilized for three days. These eggs are available from the USDA research labs in Rockville, Maryland. Place them on their side in a chicken egg incubator, and gently rock for 4 hours.
2. Remove the eggs from the incubator and gently crack out the embryo into a 3 mL petri dish. Be sure that the embryos remain intact and the yolks do not break.
3. Let the embryos sit in a three gas incubator at 37 degrees C for 2 days.
4. Place a 1-2 millimeter disk of dermal sheet onto the embryo. The disk should be placed somewhere close to, but not directly on, a major blood vessel. Gently use very thin nosed surgical tweezers to place the dermal section onto the CAM. Be

sure not to rupture the yolk or puncture a blood vessel. Once the dermal section is on the CAM, make sure that it adheres to the surface.

5. Let the disk sit on the embryo for at least 3 days. During the time that the disk is simply sitting, be sure to check to make sure that the embryos are still alive. Any dead embryos should be removed. Any discoloration, bleeding, vessel deformation, or other abnormality should be noted as it could be sign that the amount of VEGF in the sheets is too high.
6. After a period of 3 days, the dermal sheet should be cut out of the CAM using small surgical scissors. Roughly 1-2 mm of embryo that surrounds the dermal sheet should be removed along with the dermal sheet. The small section of CAM that includes the dermal sheet should be embedded in paraffin wax.
7. Once the dermal section and surrounding embryo is paraffin embedded, it should be mounted onto a slide and immuno-stained. The immuno-staining procedure varies from kit to kit, but the result should be that the blood vessels should appear dark purple.
8. If the staining shows many new small purple lines, then the dermal sheet has increased new blood vessel formation. If not, then the amount of VEGF, or location of VEGF microspheres in the dermal sheet, needs to be adjusted.

NOTE: All of the experiments above are for one person. One person running CAM tests can, assuming an eight hour work day, can manage 35 CAMs. This means that one person can run 35 experiments per week. Multiple tests can be run concurrently.

The cell-flask tests and the CAM tests can be run concurrently.

The following is a list and description of the small animal tests to be run prior to FDA evaluation. Small animal tests should only be attempted once the cellular level tests have been successfully completed.

Nude Mice Burn Treatment

1. Take 20 nude mice, mice that lack hair, and store in one foot by half a foot cages. These mice are available from the USDA research labs in Rockville, Maryland. The mice should be housed in individual cages as to keep them from fighting and potentially harming one another. Since the mice are housed separately, the sex of the mice is unimportant.
2. The mice should be burned with a red hot piece of iron. The burn should be made between the shoulder blades, and should be about 2 cm by 3 cm. Making the burn between the shoulder blades helps keep the mice from gnawing at the wound and possibly harming the healing process. This should be done in a clean, but not sterile, environment. The lack of a sterile environment helps simulate real life wounding situations, which are not sterile.
3. Immediately after the wounding, an animal surgeon should come and apply the dermal replacement. The application of the dermal replacement should be in the same fashion as the sheet would be used in a real hospital. The wound should be kept clean and coated with colloidal silver to minimize infection.

4. The animals should be checked every 4-6 hours, everyday, to observe the healing. Any infection or failure to heal should be noted. The animals should be given continual care, similar to any burn treatment in a hospital.
5. Once the wound is ready for a finishing autograft, the evaluation of the dermal replacement is complete. The time needed to heal, any animal deaths, inflammation, slow healing, or abnormality should be noted, as it could be a direct result of a dermal sheet failure.

NOTE: Steps 1, 2, 4, and 5 can be performed by a lab technician. Step 3 must be done by an animal surgeon, who will be hired on a per test basis. One lab technician can handle the care of 20 mice. One animal surgeon, handling just the application of the dermal replacements, can handle 80 mice per day. This means that one technician can handle 20 mice per week, which will be the rate determining step. Multiple tests can be run concurrently.

Diabetic Guinea Pigs

1. Take 20 diabetic guinea pigs, guinea pigs that have diabetes. These guinea pigs are available from the USDA research labs in Rockville, Maryland. The guinea pigs should be housed in individual cages as to keep them from fighting and potentially harming one another. Since the guinea pigs are housed separately, the sex of the mice is unimportant.
2. A small wound, 1 mm by 1 mm, should be cut into the bottom of one of the guinea pigs' feet. This wound should be made in a clean, but not sterile, environment. This will simulate a diabetic ulcer. Since the wound will be on the

foot of the guinea pig, there is the possibility that the animal may gnaw at the wound and hamper treatment.

3. Three days after the wound has been made an animal surgeon should apply the dermal replacement. It is important the dermal replacement not be applied to the wound immediately. Normal diabetic ulcers generally sit, untreated, for days, and we should try to mimic this as best as possible. The wound should be cleaned prior to the application of the dermal sheet. Failure to clean the wound can result in the failure of the dermal sheet adhesion. The treatment of the wound should be concurrent with the normal treatment of diabetic wounds.
4. The animals should be checked every 4-6 hours, everyday, to observe the healing. Any infection or failure to heal should be noted. The animals should be given continual care, similar to any diabetic ulcer treatment in a hospital.
5. Once the wound is ready for a finishing autograft, the evaluation of the dermal replacement is complete. The time needed to heal, any animal deaths, inflammation, slow healing, or abnormality should be noted, as it could be a direct result of a dermal sheet failure.

NOTE: Steps 1, 2, 4, and 5 can be performed by a lab technician. Step 3 must be done by an animal surgeon, who will be hired on a per test basis. One lab technician can handle the care of 20 guinea pigs. One animal surgeon, handling just the application of the dermal replacements, can handle 80 guinea pigs per day. This means that one technician can handle 20 guinea per week, which will be the rate determining step. Multiple tests can be run concurrently.

The nude mice tests and the diabetic guinea pig tests can be run concurrently.

The following is a list and description of the large animal tests to be run prior to FDA evaluation. Large animal tests should only be attempted once the small animal tests have been successfully completed.

Pigs

1. Take 5 pigs (3 year old males and females). These pigs are available from the USDA research labs in Rockville, Maryland. The pigs should be housed in individual pens or cages (approximately 3 ft long, 2 ft wide and 3 ft high) as to keep them from fighting, potentially harming one another, or breeding. It is important to have both sexes of the animals. This will allow for an evaluation on both sexes in a fashion and animal model that closely resembles humans.
2. The pigs should be burned with a red hot piece of iron. The burn should be made between the shoulder blades, and should be about 30 cm by 30 cm. Making the burn between the shoulder blades helps keep the pigs from gnawing at the wound and possibly harming the healing process. This should be done in a clean, but not sterile, environment. The lack of a sterile environment helps simulate real life wounding situations, which are not sterile.
3. Immediately after the wounding, an animal surgeon should come and apply the dermal replacement. The application of the dermal replacement should be in the

same fashion, as the sheet would be used in a real hospital. The wound should be kept clean and coated with colloidal silver to minimize infection.

4. The animals should be checked every 4-6 hours, everyday, to observe the healing. Any infection or failure to heal should be noted. The animals should be given continual care, similar to any burn treatment in a hospital.
5. Once the wound is ready for a finishing autograft, the evaluation of the dermal replacement is complete. The time needed to heal, any animal deaths, inflammation, slow healing, or abnormality should be noted, as it could be a direct result of a dermal sheet failure.

NOTE: Steps 1, 2, 4, and 5 can be performed by a lab technician. Step 3 must be done by an animal surgeon, who will be hired on a per test basis. One lab technician can handle the care of 5 pigs. One animal surgeon, handling just the application of the dermal replacements, can handle 20 pigs per day. This means that one technician can handle 5 pigs per week, which will be the rate determining step. Multiple tests can be run concurrently. Given the size of the pigs care, maintenance, and handling will be more intense.

Diabetic Dogs

1. Take 5 diabetic dogs, dogs that have diabetes, (3-5 year old males and females). These dogs are available from the USDA research labs in Rockville, Maryland. The dogs should be housed in individual pens or cages (approximately 3 ft long, 2 ft wide and 3 ft high) as to keep them from fighting, potentially harming one

another, or breeding. This will allow for an evaluation on both sexes in a fashion and animal model that closely resembles humans.

2. A small wound, 2 mm by 2 mm, should be cut into the bottom of one of the dogs' feet. This wound should be made in a clean, but not sterile, environment. This will simulate a diabetic ulcer. Since the wound will be on the foot of the dog, there is the possibility that the animal may gnaw at the wound and hamper treatment.
3. Three days after the wound has been made an animal surgeon should apply the dermal replacement. It is important the dermal replacement not be applied to the wound immediately. Normal diabetic ulcers generally sit, untreated, for days, and we should try to mimic this as best as possible. The wound should be cleaned prior to the application of the dermal sheet. Failure to clean the wound can result in the failure of the dermal sheet adhesion. The treatment of the wound should be concurrent with the normal treatment of diabetic wounds.
4. The animals should be checked every 4-6 hours, everyday, to observe the healing. Any infection or failure to heal should be noted. The animals should be given continual care, similar to any diabetic ulcer treatment in a hospital.
5. Once the wound is ready for a finishing autograft, the evaluation of the dermal replacement is complete. The time needed to heal, any animal deaths, inflammation, slow healing, or abnormality should be noted, as it could be a direct result of a dermal sheet failure.

NOTE: Steps 1, 2, 4, and 5 can be performed by a lab technician. Step 3 must be done by an animal surgeon, who will be hired on a per test basis. One lab technician can handle the care of 5 dogs. One animal surgeon, handling just the application of the dermal replacements, can handle 20 dogs per day. This means that one technician can handle 5 dogs per week, which will be the rate determining step. Multiple tests can be run concurrently.

Once large animal tests have been completed, the company is now ready to move onto the FDA evaluation process.

The animal experiments will be conducted in sets. The table below illustrates the number, type, and cost of each set of experiments.

Table 7: Number of Successful Tests Run in Each Pre-FDA Testing Set

	Number of Cell-Flask Tests	Number of CAM Tests	Number of Nude Mice Tests	Number of Diabetic Guinea Pig Tests	Number of Pig Burn Tests	Number of Diabetic Dog Tests
Set A	100	100	100	100	100	100
Set B	100	100	50	50	50	50
Set C	50	50	50	50	25	25

In order to properly evaluate which options to choose, both in the number of personnel to hire and the number of experiments to run, a risk simulation was created in excel. The full risk simulation is available. Below, on the next three pages, are three risk curves that help to illustrate the choices that were made by RepliDerm Inc.

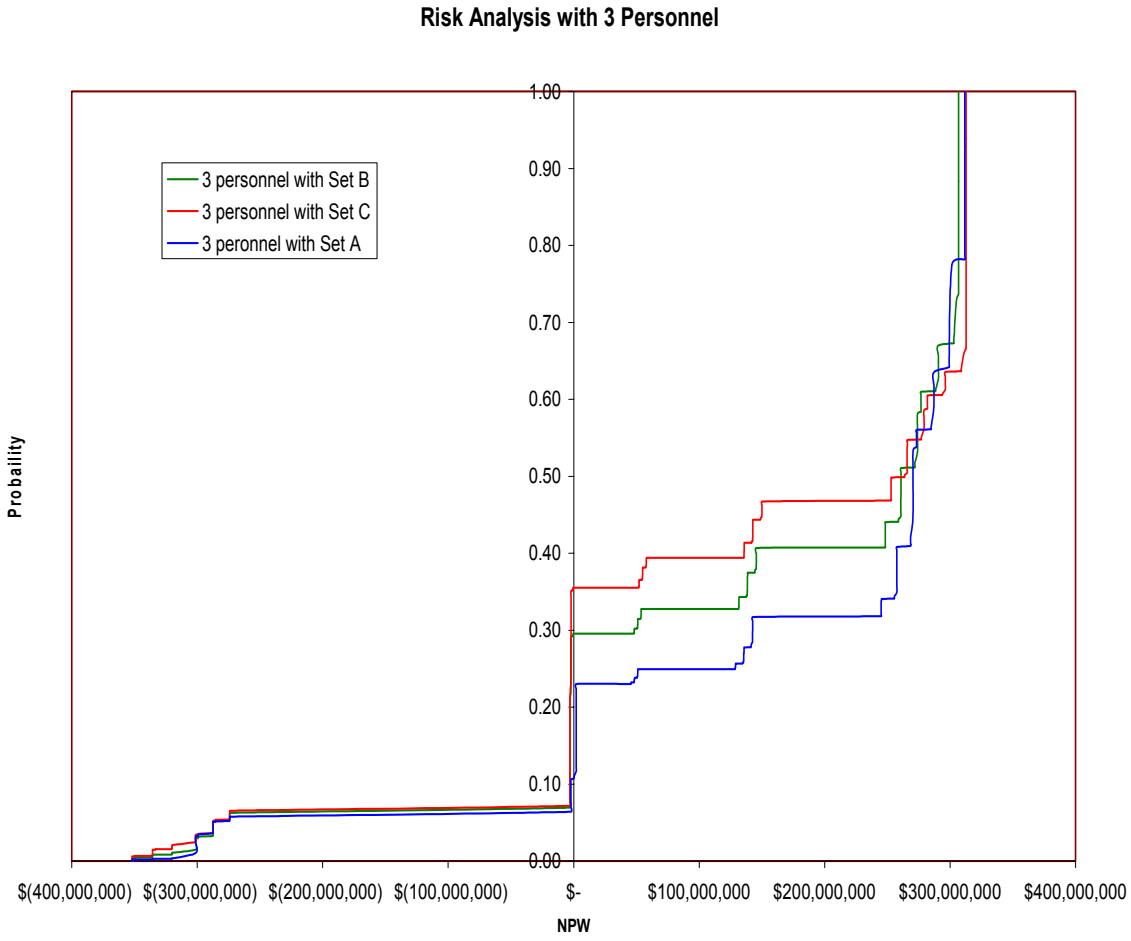


Figure 6: Risk Curves for all experiment sets with 1 Ph.D. and 3 Technicians

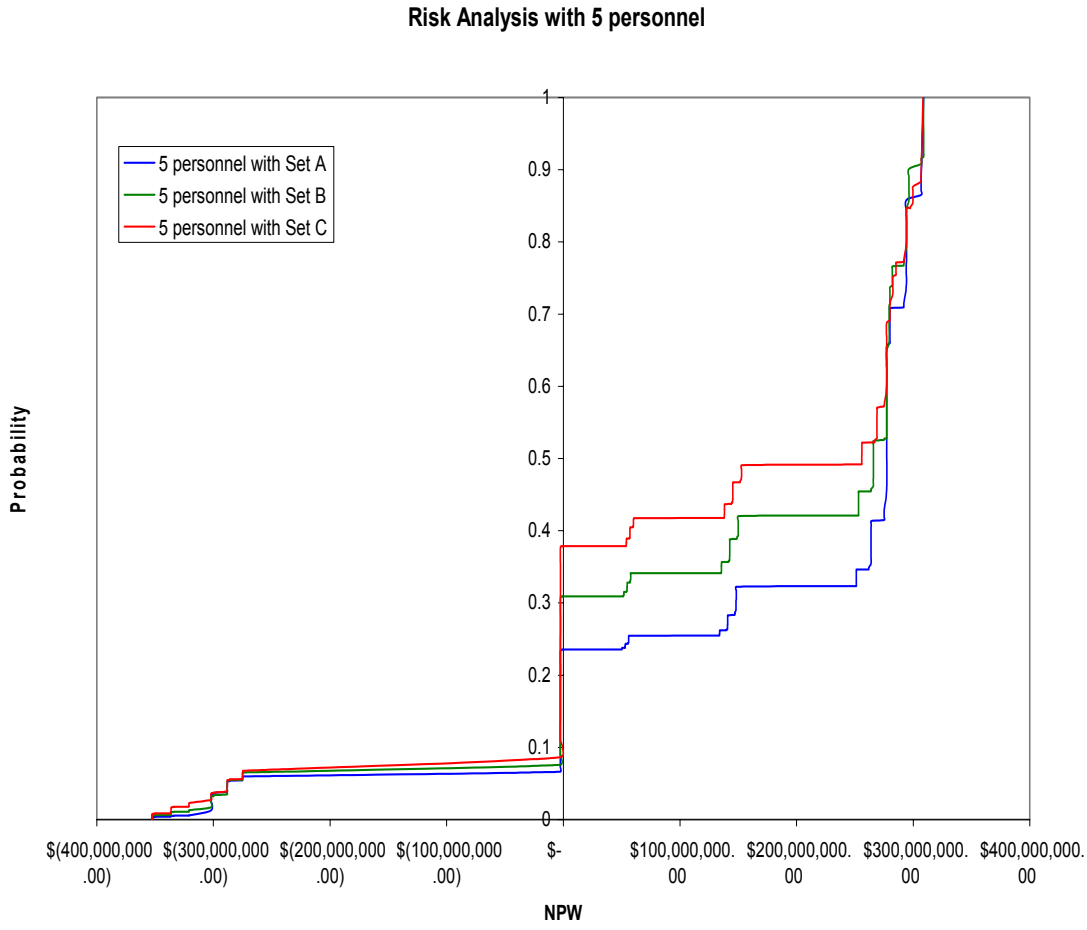


Figure 7: Risk Curves for all experiment sets with 1 Ph.D. and 5 Technicians

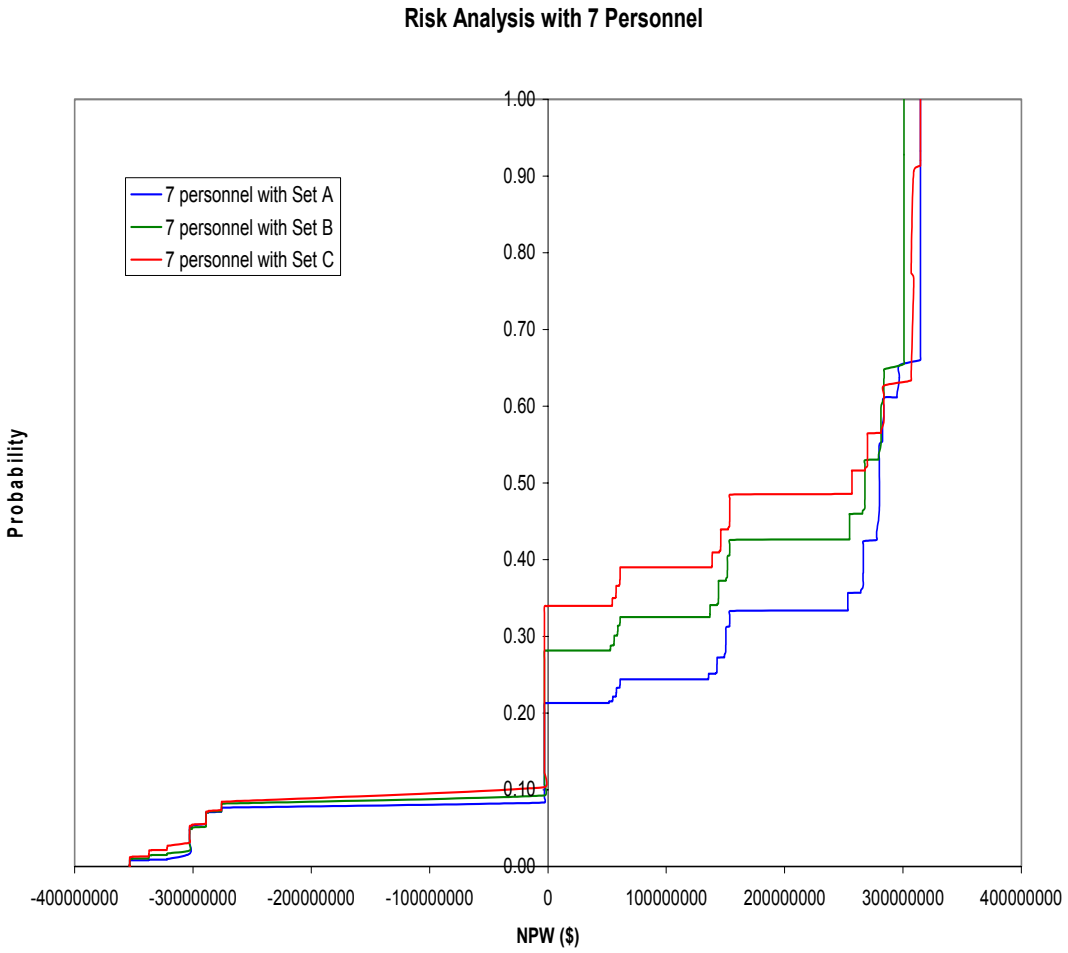


Figure 8: Risk Curves for all experiment sets with 1 Ph.D. and 7 Technicians

From the risk curves, it was determined that the experiment set providing the lowest risk and the highest probability of passing the FDA evaluations on the first try is experiment set A. Experiment set A provides for a 78% chance of success in the FDA trials, while experiment set B has a 69% chance of success and experiment set C has a 61% chance of success. Experiment set A is chosen because it provides the greatest chance for passing the FDA approval process in the least amount of time.

From the risk curve shown below, RepliDerm Inc. was able to choose the optimum number of employees to hire.

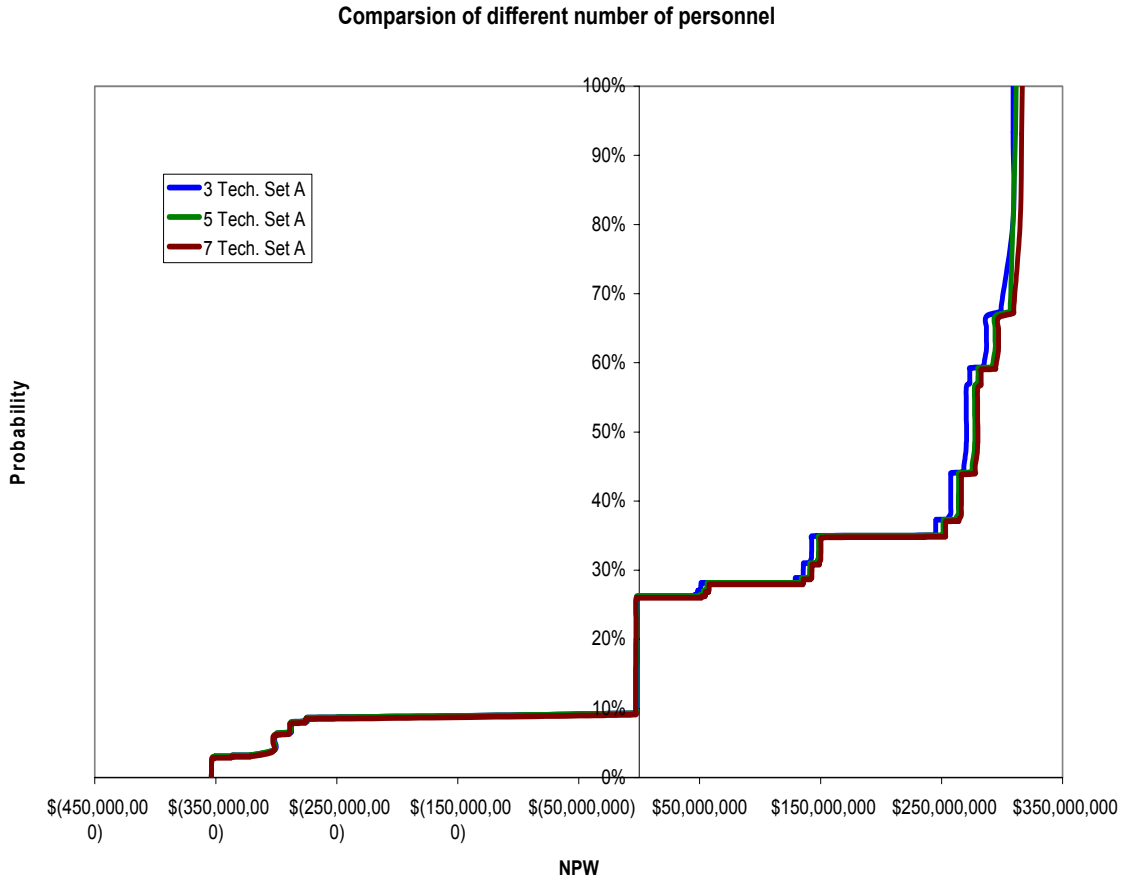


Figure 9: Risk Curves for Experiment Set A with All Employment Options

The curve above shows that seven lab technicians allow RepliDerm Inc. to make the most money possible. All three employment options (three, five, and seven lab technicians) have the same amount of risk; however, seven lab technicians will allow RepliDerm Inc. to bring their product to market quicker and therefore make more money.

Module 1 – Non-clinical Laboratory Testing

The results from Module 1 of the FDA approval process are obtained from non-clinical testing. The results from such tests are obtained from laboratory studies that involves chemical testing, biocompatibility or toxicity testing and animal and biological testing. The continuation of this module is further studied to test the sterilization, shelf life and packaging information. The period of testing for these tests lasts about 2 years. The time for these tests, 2 years, is an average of the amount of time spent developing similar products for the FDA[3].

In module 1, the efficiency and efficacy of our finished RepliDerm sheets will be tested in stages. These stages will be able to monitor the use of the dermal replacements at the cellular level, the small animal level, and the large animal level. The stages should progress in cellular complexity. Subsequent stages should only be tried once the preceding stage has been proven effective. If at any point the current stage is proven unsuccessful, then various re-evaluations must be conducted to determine the root cause of the failure and possible corrective actions.

Stage One of the FDA Evaluation:

The first stage of testing is determining RepliDerm's compatibility with cells. Since these sheets will serve as dermal replacements, initial tests should be conducted in vitro using multiple lines of commercially available human dermal cells. These cells are available from various companies, including Sigma-Aldrich and Cellgro. The sheets should be prepared according the standard production method laid out in the preceding pages. After preparation, the sheets should be cut to size to fit into a 3 mL petri dish, re-

hydrated, and placed into a 3 mL petri dish which is approximately 15% confluent with human dermal cells. A confluency of 15% will allow for cell growth at a controllable pace, and should help to elucidate the growth potential of the dermal replacement. All of this should be done in as sterile an environment as possible, and all application of the dermal replacement sheets should be done inside a cell culture hood.

Once the dermal sheet is placed inside the cell growth flask, the cells should be allowed to grow for a period ranging from 14 to 28 days. The cells should be regularly checked and fed while growing in a sterile incubator at 37°C. The conditions of the incubator should be set to mimic the growing conditions found inside the human body as best as possible. At the end of the 14 to 28 day period, the cells should be visually inspected to check proper growth and fully assayed to determine the quantitative growth rate and cellular uptake of VEGF. If this first stage of laboratory testing is successful, then it is safe to proceed to stage two. If this stage is not successful, then the production of the dermal replacement should be reevaluated.

Various points of failure for the production process could be the production of the collagen matrix, production of the VEGF microspheres, sterility, cellular compatibility, or a host of other unforeseeable errors. Errors that happen as a result of the production of either the collagen matrix or the VEGF microspheres will cause serious reconsiderations of the production method and materials used in creating the dermal replacement sheets. Errors that happen as a result of the sterility of the sheets or the sterility of the test cells will cause some adjustments to the aseptic techniques employed. This should be a relatively easy error to correct, but challenges could arise. Cells are sometimes particular about growth, and the source of abnormal growth cannot be determined. This is

especially true when human cells are grown *in vitro*. Perhaps the dermal replacement sheets are satisfactory; however, the cells are also very difficult to grow. Should this happen, it would be a matter of fitting the testing to the cell growth. Other unforeseeable errors would cost far more and cause far more perplexing challenges than those stated. A chart outlining potential failures, solutions, probabilities of failure, and the cost of those failures is on the following page.

Table 8: Stage One Failures

Failure	**Causes**	**Probability of Failure**	**Cost to Fix Failure**
Collagen Matrix	Poor Production Method	5%	$1000
	Poor Collagen quality	10%	$1500
	Poor Sterilization of Collagen	10%	$2000
VEGF Microspheres	Poor Production Method	10%	$3000
	Poor Source of VEGF	5%	$5000
	Poor Sterilization of Microspheres	10%	$2000
Assembly of Sheet	Poor Dispersion of Microspheres	15%	$2500
	Poor Timing in Microspheres Added	5%	$2500
Use of Silicone Sheet	Poor Source or Type of Sheet	2%	$1000
Overall Sterilization	Poor Sterilization Technique	20%	$10000
	Poor Aseptic Technique or Handling of Sheet	5%	$1500
Cell Failure	Poor Line of Cells	15%	$5000
	Poor Cell Handling	30%	$4000
	Poor Cell Growth Conditions	35%	$6000
Unforeseen	Causes Vary	50%	$10000

Stage Two FDA Evaluation:

The second stage of testing should consist of determining the RepliDerm's compatibility with small living animals. This stage should be conducted with relatively small and easy-to-manage animals. These animals will have to mimic the effects of the intended purpose of the product. The animals used will most likely be rats or mice. Rats and mice are small, easy to handle, inexpensive, and readily available for a wide variety of testing methods. For the burn patients, the rats will have to have artificial wounds created. It will also help if the rats are hairless. To facilitate this, nude mice should be chosen. The nude mice lack hair and have a surface on which it is easy to create a burn. Specific techniques for creating wounds in animals vary from location to location.

To attempt to meet most states' requirements, the mice should be burned in a clean environment (not sterile) using a small branding iron or heated rod. The brand should be left in place long enough to require the area to need a graft. The mice would then be treated with our dermal replacement by an animal surgeon in a fashion similar to the manner in which an actual surgeon would apply the graft. The animal surgeon employed to perform this operation will be contracted to perform these experiments on an as needed basis. The mice should be monitored regularly (at least four times a day). Their treatment should be consistent with the treatment that a human patient would receive. The wound should be kept clean, treated with silvadien (a colloidal silver solution used to minimize infections in wounds), antibiotics should be administered as needed, and life necessities of the mice should be met. At the end of the healing process, the mice should be qualitatively inspected as to the quality of the wound healing, including aesthetic appearances. The mice should be quantitatively analyzed as to the

amount of successful treatments, time needed to heal, and amount of rejection, infection rate, and overall success of the grafts.

When testing is conducted for the efficacy of our graft in diabetic patients, the testing procedure will vary only in the regard that the mice will be specially bred to be diabetic-prone mice. These mice are overweight and are genetically predisposed to having elevated blood sugar levels. The wounds will be made using a scalpel to cut a small portion of the skin off the feet of the mice. The mice will then be treated with our dermal replacement in the same manner as the mice that were burned.

As with any experiment, failures can arise. If the mice develop an infection, the technique with which the graft was applied should be investigated to determine whether fault lies with the graft or its application. If the failure was with the graft, then the graft's sterility should be examined. If the mice reject the graft, then serious evaluation of the graft's biological compatibility should be investigated. The graft may not be suitable in mice, but it may be suitable in humans. If the graft contains a major flaw, like the stimulation of a dangerous inflammation or fever, then the graft needs to be reworked to eliminate this. If some mice do not heal properly but the graft has been seen to be effective in other specimens, the flaw might simply be in the animal surgeon administering the graft. These tests are conducted in an effort to eliminate all possible failures prior to FDA evaluation.

Table 9: Stage two failures

Failure	Causes	Probability of Failure	Cost to Fix Failure
Mice Die	Poor Diet	10%	$500
	Poor Living Conditions	25%	$3000
	Defective method of Applying Graft	45%	$45000
	Poor Wounding Technique	30%	$2000
Infections	Poor method of applying Graft	50%	$50000
	Poor Living Conditions	25%	$3000
	Ineffective Surgery	25%	$10000
Poor Healing	Poor method of Applying Graft	30%	$15000
Unforeseen	Causes Vary	30%	$25000

Stage Three FDA Evaluations:

The third stage of the testing should examine the interaction of the dermal replacement sheets with large animals. The large animals should be of greater biological complexity than the rats and/or mice used in the second stage. The large animals should also try to mimic the physiology of humans as much as possible; this would allow for as smooth a transition into human clinical trials as possible and would help eliminate any possible complications before they appear in human trials. It is for all these reasons that pigs would be the most logical test animals. Pigs have a very similar genetic make up to human beings. Their skin has many properties similar to that of humans: it is prone to burn, it has hair follicles, and sweat and sebaceous glands lay fairly close to the surface. Pig skin is sometimes used for temporary burn coverage in emergency situations. Pigs have served as organ donors for humans in the past, thus giving a precedent to justify the close physiological relationship between humans and pigs. They are also relatively cheap and plentiful.

The creation of the wound would be similar to the method used in the stage two study. The pig would be burned using a branding iron or some sort of heated metal rod. The skin would be burned to the point that it necessitated the use of a graft. An animal surgeon would then place a graft onto the pig in a manner similar to that of a surgeon placing the graft onto a human. For the evaluation of the efficacy of our graft in diabetic patients, the procedure will vary only in the fact that a wound will be created on the feet of the pigs. A small section of skin will be cut away, and the wound will be treated with our dermal graft.

After the operation, the pigs will be monitored regularly (at least four times a day) for a period until the wound is fully healed. The pigs will be evaluated qualitatively as to the aesthetics and quality of the healing. The pigs will also be quantitatively analyzed as to the amount of successful treatments, time needed to heal, and amount of rejection, infection rate, and overall success of the grafts.

As with any experiments, failures can arise. If the pigs develop an infection, the technique with which the graft was applied should be investigated to determine whether fault lies with the graft or its application. If the failure was with the graft, then the graft's sterility should be examined. If the pigs reject the graft, then serious evaluation of the graft's biological compatibility should be investigated. Pig rejection of the graft could mean serious rejection issues in human patients. Since this is the last trial before the graft is tested in humans, it is absolutely necessary to eliminate as much potential for rejection as possible. Of course there is the possibility that the graft may just not be suitable to pigs, but it may be suitable in humans. If the graft contains a major flaw, like the stimulation of a dangerous inflammation or fever, then the graft needs to be reworked to

eliminate this. If some pigs do not heal properly but the graft has been effective in other specimens, then flaw might simply be in the animal surgeon administering the graft. As with the tests involving the small animals, these tests are designed to eliminate any possible failures that may arise prior to FDA evaluation.

Table 10: Stage Three Failures

Failure	Causes	Probability of Failure	Cost to Fix Failure
Pigs Die	Poor Diet	25%	$2500
	Poor Living Conditions	40%	$5000
	Defective method of Applying Graft	50%	$60000
	Poor Wounding Technique	30%	$12000
Infections	Poor method of applying Graft	50%	$70000
	Poor Living Conditions	40%	$5000
	Ineffective Surgery	30%	$15000
Poor Healing	Poor method of Applying Graft	50%	$20000
Unforeseen	Causes Vary	30%	$80000

In this study, any of the listed failure possibilities will lead to disapproval of the module. Invariably, this means a higher cost for the lab trials.

Assuming all testing goes according to planned. The estimated cost of the non-clinical laboratory testing would be about $500,000. RepliDerm Inc. will hire a professional PhD for most technical and process development purposes. Personnel cost are the highest in the non-clinical trials, and this is justified in that it is important to have a reliable, trained and dedicated staff during this period because data and analysis don in this period need to be consistent. The table below shows the general breakdown of the non-clinical trial expenses for 2 years.

Table 11: Non-Clinical Cost for 2 year period

	Cost /yr	**Cost in 2 yrs**
Cost of sterilization Equipments + Installation		$2000
Equipment cost (balances…..)		$12000
Refrigeration		$10000
Administrative Materials (computers…)		$5000
Total Fixed Capital Cost		*$29,000*
1 Professional Consultants (PhD in Biomedical Science)	$100,000	$200,000
3 Lab assistants ($35,000/person)	$105,000	$210,000
1 Part time animal surgeon	$30,000	$60,000
Fringe Benefits (health care, retirement, etc.)	$20,500	$41,000
Small Animals (rats)	$5,000	$10,000
Large animals (rats)	$10,000	$20,000
Other Operating costs (rent, refrigerant, pipettes, maintenance….	$15,000	$30,000
Total Operating Costs	*$25,000*	*$600,000*
Total Cost		**$629,000**

Shown on the following page is a flow diagram illustrating the paths available for Module 1. For this flow sheet, experiment set A was performed.

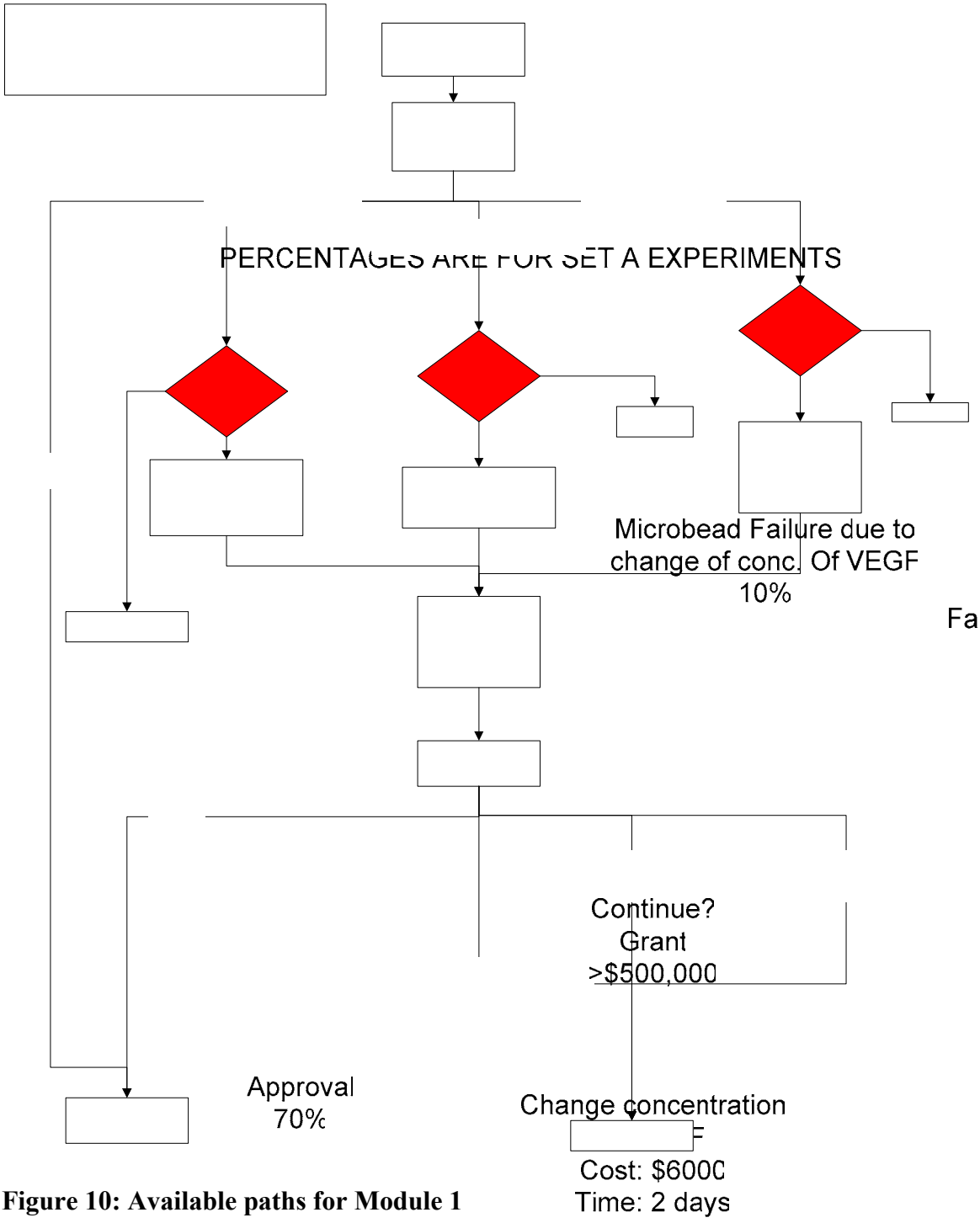


Figure 10: Available paths for Module 1

Appendix A contains all of the possible decision and Pert charts that could happen. The percentages of various failures and passing the FDA will depend on the experiment set chosen.

Module 2 – Quality System and Inspections

Module 2 involves detailed manufacturing information of the product. The FDA requires that domestic or foreign manufacturers have a quality system for the design, manufacture, packaging, labeling, storage, installation, and servicing of finished medical devices intended for commercial distribution in the United States. Some regulations have therefore been set up by FDA known as Good Manufacturing Practices (GMP) or Quality System (QS) Regulations. The QS regulation helps assure that medical devices are safe and effective for their intended use .In order to be approved by the FDA in module 3. The following needs to be considered:

- The production processes should be developed, conducted, controlled, and monitored to ensure that a device conforms to its specifications.
- Management need to provide adequate resources like trained personnel, for management, performance of work and assessment of activity. It is the company's responsibility to have sufficient personnel with the necessary education, background, training and experience with the device.
- Management needs to review the sustainability and effectiveness of the quality systems frequently at different intervals such that it satisfies the requirements at all times.
- Inspection control requires that equipments be measured, tested calibrated and maintained routinely.
- Labeling inspection requires that all labeling should be storied in manner that provides proper identification to prevent mix-ups. Also labels should have correct

expiration dates, handling instructions, storage instructions, a control number and additional processing instructions.

- Device packaging should be constructed to protect the device from damage and alterations
- Storage procedures should be established and maintained to prevent mix-ups, damage, deterioration, contamination, or other adverse effects pending use or distribution and to ensure that no obsolete, rejected, or deteriorated product is used or distributed.
- Post approval inspection is performed about 8-12 months of approval

Inspection will include an assessment of the firm's capability to design and manufacture the device as claimed in the submitted PMA All required information and procedure in the Quality Systems and Good Manufacturing Practices are further discussed in our business organization.

Shown below is a flow diagram illustrating the paths available for Module 2. For this flow sheet, experiment set A was performed.

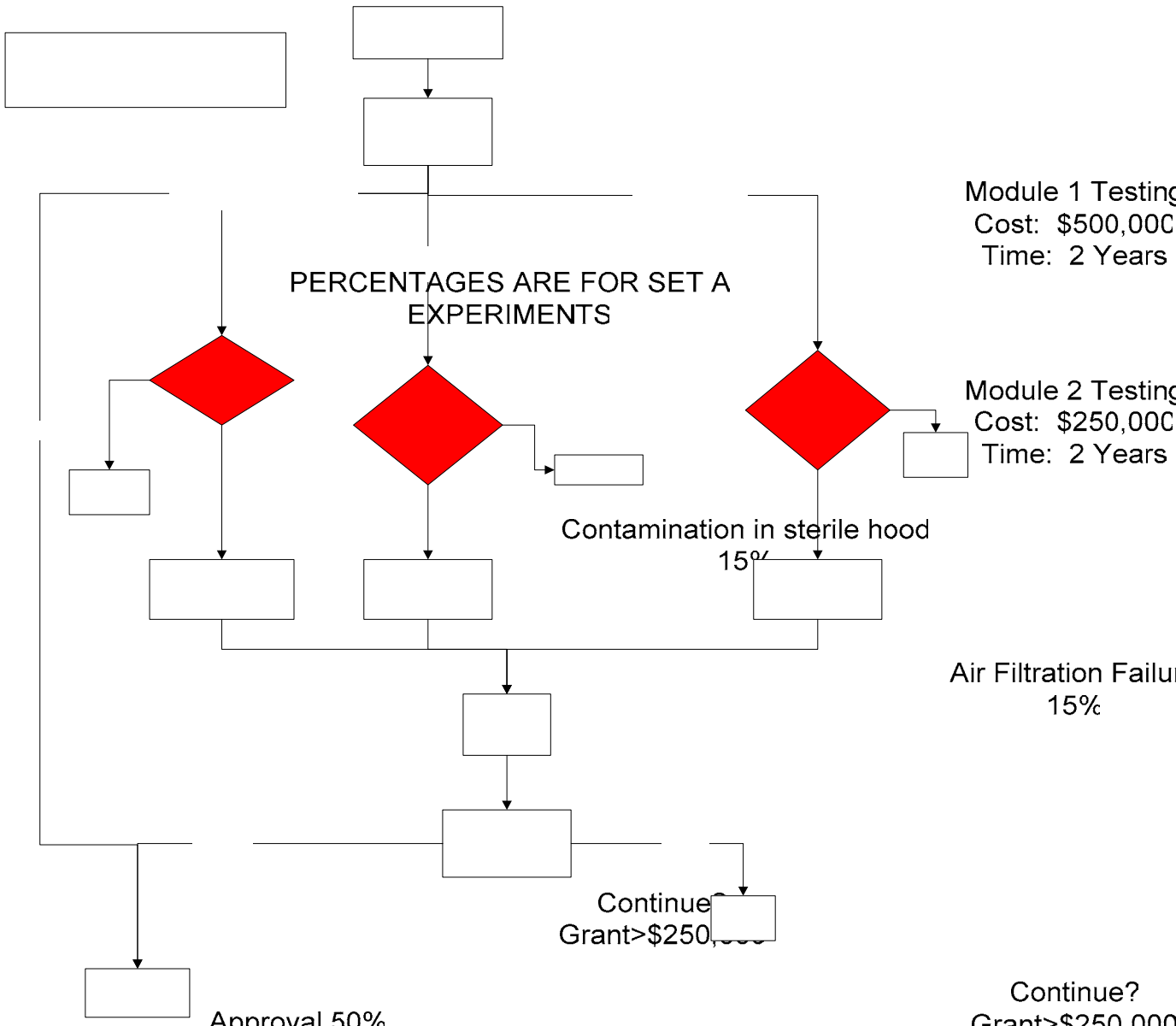


Figure 11: Available paths for Module 2

Module 3 - Clinical Trials

Once all manufacturing processes have been studied and fitted such that it satisfies the Good Manufacturing Practices of the FDA, the next stage is the Module 3. This is the final module in which all the clinical data, financial disclosure information, instruction for surgeons, and operation manual is completed. This is not only the most

expensive phase, but it is also the longest phase in the PMA approval process. It took Integra 17 years to go through clinical trials. It has been estimated that it will take RepliDerm about 10 years to go though clinical trials. This time frame assumes that all of the trials are successful on the first try.

Clinical Trials

Our clinical trials will be preformed using 900 patients over a period of ten years. The clinical trials will be done on 450 Burn patients and 450 Diabetic Ulcer patients. The FDA will randomly select the hospitals that will receive RepliDerm Inc.'s product for evaluation and use. Once delivered to the hospital, the surgeons performing the operations will administer RepliDerm in the standard procedures for the various patients. The cost to perform clinical trial studies per patient was averagely estimated to be $760,000 per burn patient and $20,000 per diabetic ulcer patient. As mentioned earlier, each sheet covers an area of 6 inches by 4 inches. A burn patient will require an average of 12 sheets for healing. A diabetic patient will require an average of 1 sheet for healing. In clinical trials, the company undergoing the trials will be responsible for covering the cost of the volunteer patients who participate in the trials. A burn patient has an average cost of $10,000 a day for hospital. Hospital care usually lasts 10 days. The cost of the surgeons and any added antibiotics and medicine leads to another $660,000 per day. A diabetic ulcer patient usually requires one sheet. Hospital stays usually last one day at a cost of $5,000 a day. The hospital usually administers medication and charges for the use of a surgeon to clean the wound and apply the dermal sheet. This adds another $15,000 to the patient's bill. It costs more to perform a clinical surgery on a burn patient because

on average, twelve sheets of RepliDerm are used per burn victim and one sheet per Diabetic Ulcer patient.

The cost for Clinical trial is estimated to be **$351,000,000.** The costs are broken down in the table below.

Table 12: Clinical Cost period

	Cost /yr	**Total Cost**
450 Burn Patients($760,000 / patient)		$342,000,000
450 Diabetic Ulcer Patients ($20,000 / patient)		$9,000,000
1 Professional Consultants (Ph.D.)	$100,000	$100,000
3 Lab assistants ($35,000/person)	$105,000	$105,000
Operating cost of *RepliDerm* Production (refrigerant, pipettes, maintenance….		$2,600,000
Total Cost		**$353,805,000**

Shown on the next page is a flow diagram illustrating the paths available for Module 3. For this flow sheet, experiment set A was performed.

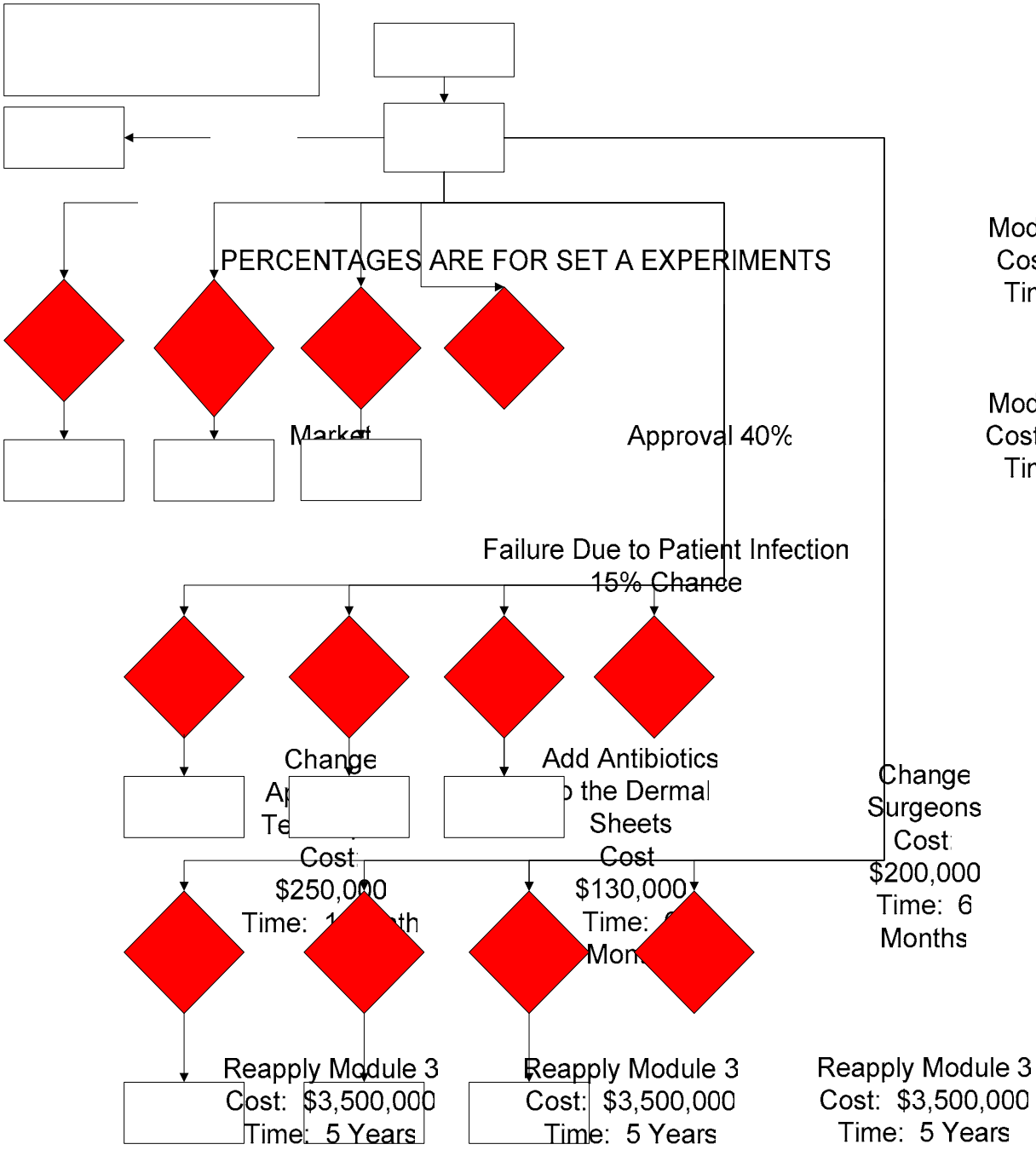


Figure 12: Available paths for Module 3

The reported costs are estimated based on the assumption that the FDA approves the product without a hitch in the approval process. The probability that this occurs is not high. As a result, the cost for clinical trials will be higher than this estimation.

Trials must be performed where patients are located. According to research, the most viable resources for clinical trials are private clinical research centers.

Marketing departments prefer that clinical studies be conducted by recognized names at recognized institutions, but it has been observed that many manufactures tend to target these big institutions at the same time, which creates a scenario in which manufactures fight for marketing territory. Therefore, it is more reasonable to target yet-to-be-established sources rather than established ones because they will be are easier to work with and deliver a more reliable product.

Contract research organizations add a layer of bureaucracy between the device manufacturer and the patient. The ethics of having a clinical research center monitor the work done by its investigator-partner may be questionable. As a result, RepliDerm Inc. has decided to target managed care organizations and outpatient clinics. Research shows that patients will tend to trust these sources more because they tend to prefer to receive care in smaller clinics and hospitals rather than large centers. Also, clinics tend to support these clinical trials, so it will be easy to find a good source for trials. RepliDerm Inc. will also solicit for more patients by regularly adverting in journals, exhibiting in conferences, and making contacts with possible buyers.

FDA Approval Summary

The FDA approval process is separated into three major modules. Module 1 involves the non-clinical laboratory testing. This module will be done in 3 stages by RepliDerm Inc. The first stage will test be in-vitro testing of the template. The second stage is the testing on small animals and the third stage is testing on large animals.

Module two is the inspection of all processes and practices by the FDA. Module 3 involves Clinical Studies. It is estimated that all three modules will be carried out in a total period of 15 years.

The total cost for FDA approval is $57,854,000 assuming all stages are approved once by the FDA. FDA may deny approval of a PMA for any of the following reasons:

- The PMA contains a false statement of material fact.
- The device's proposed labeling does not comply with the requirements in labeling, or In Vitro Diagnostic Products for Human Use.
- The applicant does not permit an authorized FDA employee to inspect the facilities and controls in which the device will be manufactured or to have access to and to copy and verify all records pertinent to the application
- An essential non-clinical laboratory study described in the PMA was not conducted in compliance with the good laboratory practice regulations in and no reason for the noncompliance is provided, or, if it is, the differences between the practices used in conducting the study and the good laboratory practice regulations do not support the validity of the study.
- Any clinical investigation involving human subjects described in the PMA that is subject to the Institutional Review Board regulations and was not conducted in compliance with these regulations such that the rights or safety of human subjects were not adequately protected.

Business Organization

Business Goal:

Our goal is the production of new allograft RepliDerm by RepliDerm Inc. for the treatment of burn victims. The initial objective is to obtain research grants for research and development of RepliDerm. The figure below shows our major competitors and the share of market they hold. Our major competitors in skin tissue replacement are LifeCell's Alloderm, Genzyme's Epicel and LifeScience's Integra.

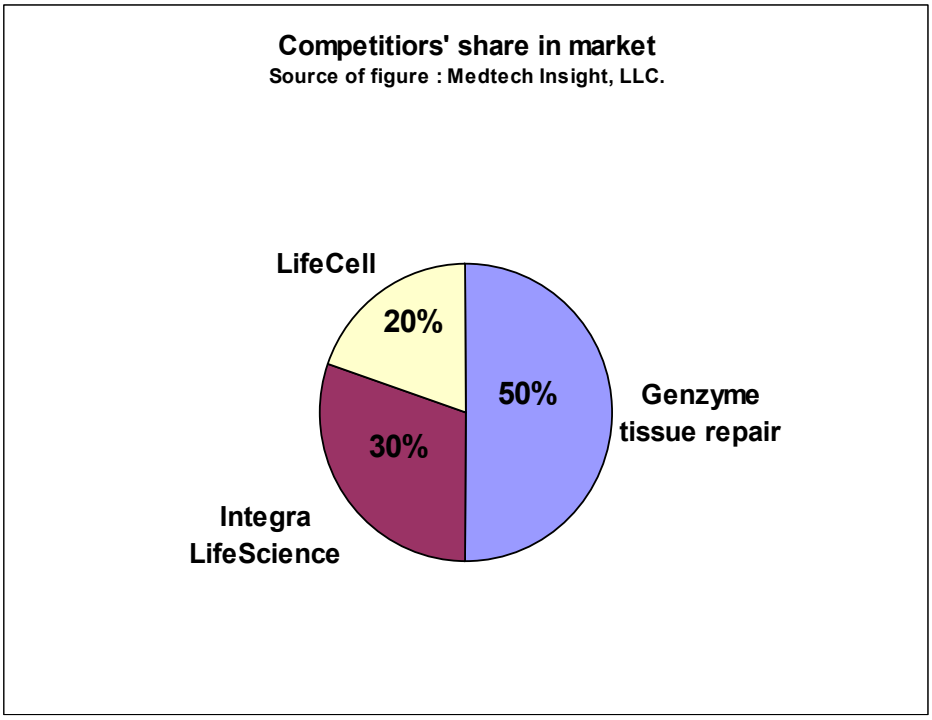


Figure 13: Market competitors

The major candidates for obtaining funds for our project will be the National Institute of Health (NIH), Small Business Innovation Research Program (SBIRP), and from Small Business Technology Transfer Research (STTR). Our initial production rate will be 2,200 sheets per month (from the developed economic model).

General Information:

The majority of our initial work will be done in laboratory research. After promising results are obtained, RepliDerm Inc. will apply for government grants for further research and development. Once RepliDerm Inc. has conducted sufficient research and development, we will submit our proposal to the U.S. Food and Drug Administration (FDA) and proceed with phase 1 testing of the FDA approval process. After completing the first phase of the FDA approval process, major sources of funding will be needed because phase 2 and 3 of the FDA approval process are extremely costly. We expect the entire FDA approval process to last for roughly 15 years.

Location of RepliDerm Inc.

Several locations were considered for the headquarters of our operation. The list of originally selected states is given below.

- New Jersey
- California
- New York
- Pennsylvania
- Texas
- Massachusetts
- Maryland
- Florida
- Ohio
- Michigan

The evaluation for the best headquarters location was done by defining important criteria (cost of living, population base, etc.) and evaluating each location under the criteria. NIH funding to each state was given a 30% importance in our decision making process. Employment, number of private biotech companies, cost of living, the number

of surrounding hospitals, and corporate tax rates were given a 20%, 15%, 15%, 10% and 10% importance respectively.

Table 13: Ranking of each state for the evaluation

Location	NIH funding	No. of Hospitals	Private Biotech companies	Cost of living	Employment in Biotech companies	Corporate Tax	Rank
Importance	0.3	0.1	0.15	0.15	0.2	0.1	
New Jersey	1	1	7	8	9	3	4.8
California	10	10	10	10	10	4	9.4
New York	8	8	8	9	8	5	7.9
Pennsylvania	7	7	6	6	7	1	6.1
Texas	5	9	9	2	6	9	6.2
Massachusetts	9	3	5	7	5	2	6.0
Maryland	6	2	4	1	4	6	4.2
Florida	2	6	3	5	3	8	3.8
Ohio	4	5	2	3	2	7	3.6
Michigan	3	4	1	4	1	10	3.3

The ranking of each state was done on the scale of 1 (lowest) to 10 (highest). The rank of each state was multiplied with the relative importance (a percentage) of the criteria to determine the "rank" column shown above. According to this method, California was determined to be the best choice of location with the highest rank of 9.4. Because California has so many advantages in comparison to the other locations, we chose Fairfield, California as our operation headquarters.

Shipping and Transportation

To prepare RepliDerm for storage and shipping, it is frozen at -80°C and then vacuum sealed in a sterile foil wrapper. The product is then packed up to 50 lbs in dry ice. In order to deliver our product to burn/wound treatment centers as fast as possible, our company will use Federal Express (FedEx) to deliver it. The cost of overnight delivery with thermal control is roughly $185. FedEx shipping can ship the product overnight to any location in the US from California for $185 or less (depending on the shipping location). If RepliDerm were based out of the New England area, shipping rates will be as high as $340 and overnight shipping of the product would not be guaranteed in all cases. Additionally, larger shipments of RepliDerm (up to 100 lbs total shipping weight) could not be shipped in sufficient time to the western US.

Advertising

After FDA approval is obtained, we plan to create a marketing and sales team to market our product with various surgeons and others who will be using our product. We will also participate in different national conferences, trade shows, and fellowship programs.

Major hospitals around the United States will be directly targeted as potential buyers for our product. The following hospitals are considered our most important potential buyers for our product:

- Shriner's Hospital-Galveston, TX
- William R. Hearst Burn Center-NY, NY
- Jaycee Burn Center-Charlotte, NC

- Arizona Burn Center-Phoenix, AZ
- U Mich. Trauma and Burn Center.-Ann Arbor, MI
- UCSF Burn Center-Fresno, CA

Table 14: Marketing cost detail

Advertising strategies	Description of cost	$ Cost
Free product distribution (1st six months)	Cost of sheets in 56 hospitals across the country considering average 5 surgeons in a hospital	$281,120.00
Free product distribution (2nd six months)	Cost of sheets in 56 hospitals across the country considering average 5 surgeons in a hospital	$281,120.00
FedEx Delivery cost to above locations	Cost of each delivery with thermal control	$16,800.00
3 National conference	One conference attendance include travel expense, hotel staying and miscellaneous cost	$6,300.00
2 International conference	One conference attendance include travel expense, hotel staying and miscellaneous cost	$11,000.00
Trade shows and fellowship	2 of each, cost estimation similar to a national conference	$6,000.00
Total annual cost of marketing		$602,340.00

The map below shows the location of the listed hospitals. The well distributed location for marketing across the United States can be easily seen.

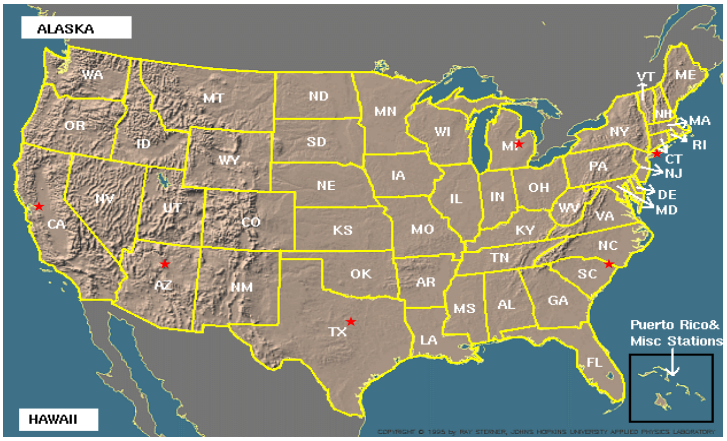


Figure 14: Location of Major Target Hospitals for Marketing/Distribution

Approximately 50 hospitals other than the major six hospitals listed above will be provided with samples of our product. Table 13 shows the estimated cost of product marketing. We will attend as many national conferences as possible for the demonstration of the effectiveness of our product.

Cost Evaluation

Table 14 shows the estimated fixed capital investment with the FDA investment included in indirect cost as legal expenses. Equipment and building costs are shown below. The cost of our production staff includes 3 technicians, 1 Ph.D. and 20 additional production workers. The major part of indirect cost is the FDA approval process- it is estimated to be $349,079,000 as shown in Table 14.

Table 15: FCI Calculation

Purchased equipment cost	$108,152.60
Installation cost	$1,000.00
Building (Including services)	$8,250,000.00
Service facilities	$600,000.00
Raw material	$7,038,000.00
Direct Cost	$8,959,152.60
Engineering and supervision	$30,000.00
Legal expenses	$30,000.00
Contingency	$60,000.00
FDA	$350,000,000.00
Indirect Cost	$350,120,000.00
Fixed Capital Investment	$359,079,152.60

In the chart on the following page, the potential market for our product is summarized. It should be noted that the following chart is of the potential and not

realized market. In essence, it assumes that we have no competition and that our product will be used to treat all wounds and burns that it is capable of treating.

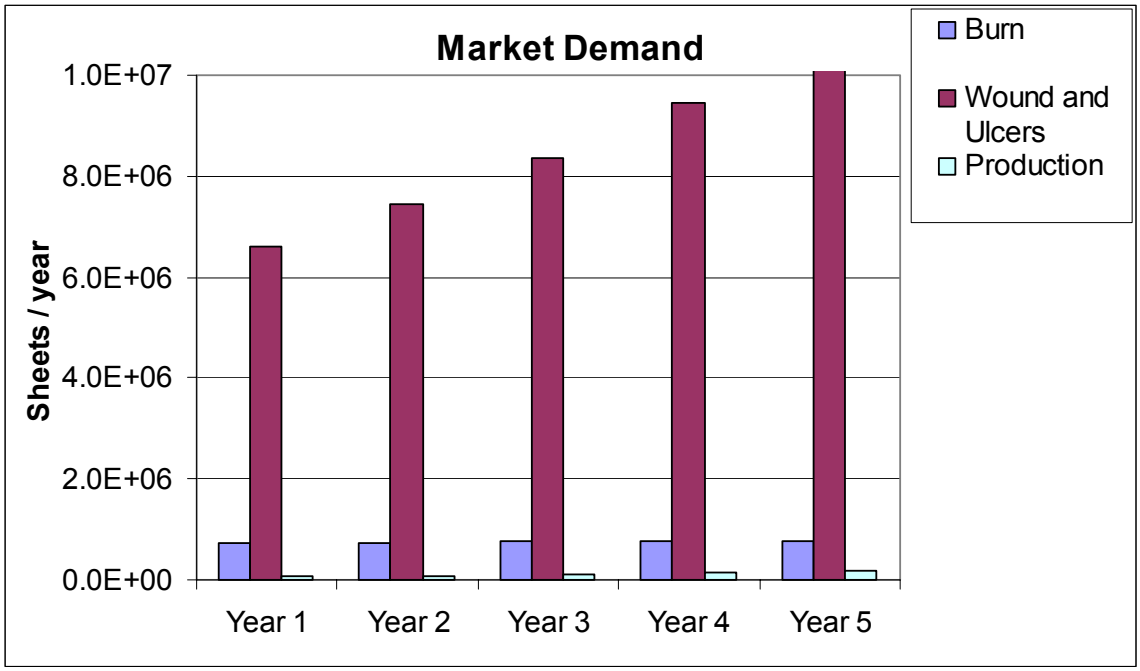


Figure 15: Total Potential Market

From available company information, LifeCell produces roughly 100,000 dermal replacement sheets per year. According the pie chart in Figure 6, LifeCell holds 20% of the total market. From this fact, we can estimate the total realized market of dermal replacement sheets to be approximately 500,000 sheets produced annually. By considering total market sales, the following model was developed to find the necessary production rate and product cost of RepliDerm.

Demand and Pricing Model

In order to determine the necessary production rate and selling price of RepliDerm, the following economic model was formulated to explain the relationship between the demanded quantity and the market price. The following equation is the most fundamental and is representative of a simplified model for the relationship between the selling price and quantity demanded. We begin the model with the following basic equation:

$$p_1 d_1 = p_2 (D - d_1)$$

In the above equation, p_1 is RepliDerm's selling price, d_1 is the production rate based off of the estimated market demand, p_2 is the average competitor's product price, and D is the total market demand.

The above equation assumes that the demanded quantity of RepliDerm is inversely proportional to the price at which it is sold. It also assumes that the price for RepliDerm, equal to that of the competitors' price, results in an evenly shared market. This would be a realistic expression of the market if the prices of the products were equal, if both RepliDerm and its competitors were in the market for a long time, if the quality of each product was the same, if advertising campaigns were equally effective for RepliDerm and for its competitors, and if production capacities of all competitors are equal.

The competitors have a clear advantage over RepliDerm because they have been established in the market for a number of years. They have earned loyal customers and have successful advertising campaigns. On the other hand, RepliDerm is a superior product which will save burn and wound centers time and money. Just as competitors

have run successful advertisement campaigns, RepliDerm will likewise be able to run such campaigns.

To account for influences from market demand and advertising on the market, two functions are introduced to the model:

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

The function α is a function of time (t) and RepliDerm's advertisement campaign (a). It represents RepliDerm's competitors' competitive advantage by virtue of their present standing in the market. α is a number between zero and one. At time=0 α is zero and RepliDerm's demand is zero no matter what the price is. Over time α should approach a limit of one.

The function β is also a function of time and RepliDerm's advertisement campaign. It represents the superiority of RepliDerm as a wound treatment and ultimately its competitive edge. At time = 0, β is equal to one (representing no initial advantage). As time increases, β approaches zero. If β could reach zero, RepliDerm's revenue would be infinite.

The graph on the following page shows values for alpha and beta that were chosen to reflect the strong foothold that the competitors enjoy in the current market and the superiority of the RepliDerm product.

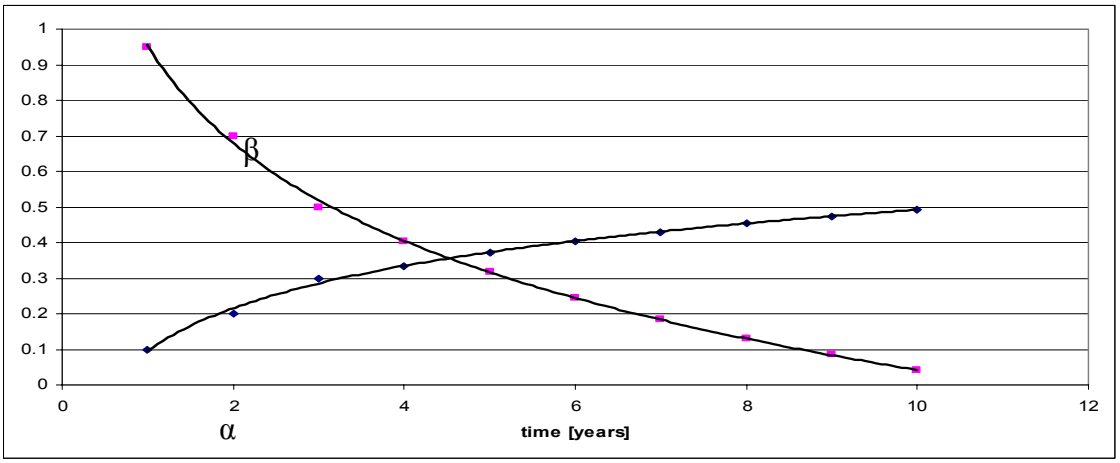


Figure 16: Values for alpha and beta as a function of time

The estimated values for alpha and beta are estimates based on the performance of similar novel products in the past. Limited access to information concerning competitor's sales creates a good deal of uncertainty in these values. It is expected that following initial sales of RepliDerm trends can be observed to better define the functions that influence the demand of the product.

A general rule of thumb, in the tissue engineering industry states that a company should try to recover their investment within two to three years after FDA approval. The science of tissue engineering and biomimetic materials is growing so rapidly that any product on the market can be expected to be replaced by a superior product within a short number of years. For this reason it is advisable to recover any incurred debt in a short amount of time.

Assuming that we will recover all investment within the first three years of operation following FDA approval, we set the cumulative cash flow equal to the FDA costs and fixed capital investment costs as shown by the following equation:

$$\sum_{i=1}^{3} p_1 d_{1i} - PC = FDA + FCI$$

In the previous equation, $PC = pc \cdot d_1$ and pc is the production cost per sheet.

Rearranging our economic model to solve for d_1 the following expression is obtained:

$$d_1 = \frac{p_2 D\alpha(t)}{\beta(t)p_1 + \alpha(t) \cdot p_2}$$

Substituting this model into our cumulative cash position equation for the first three years:

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

The current market situation is estimated as follows:

p_2=\$1000/sheet

D=500,000 sheets

pc=\$195/sheet

Solving for p_1 yields the suggested sale price of RepliDerm at \$1870/sheet. This is almost twice the cost of competitor's products. The increase in price is justifiable by virtue of the fact that RepliDerm will save hospitals time and money by allowing for a faster and therefore cheaper patient recovery.

Substituting the selling price back into the economic model the production for each year can be determined.

Table 16: Cumulative Cash Position Calculations

Year	Sale price with 2% inflation rate ($)	Rate of production (sheet/yr)	$Revenue/yr	Raw material cost ($)	Total product cost/yr	Cash flow ($)
1	1870	26600	49800000	5200000	6080000	4.37E+7
2	1870.02	67600	1.26E+08	13470000	14500000	1.12E+8
3	1870.04	126300	2.36E+08	25700000	26900000	2.09E+8
4	1870.06	162200	3.03E+08	33640000	35050000	2.68E+8
5	1870.08	208900	3.91E+08	44200000	45850000	3.45E+8
6	1870.1	258900	4.84E+08	55890000	57810000	4.26E+8
7	1870.12	313400	5.86E+08	68990000	71220000	5.15E+8
8	1870.14	373200	6.98E+08	83810000	86420000	6.12E+8
9	1870.16	439600	8.22E+08	100700000	103760000	7.18E+8
10	1870.18	514000	9.61E+08	120100000	123690000	8.38E+8

The cumulative cash position chart shows cash flow projection of 10 years after production and 15 years before the production assuming 2% inflation rate. The production growth rate will follow the model as shown in Figure 10 below.

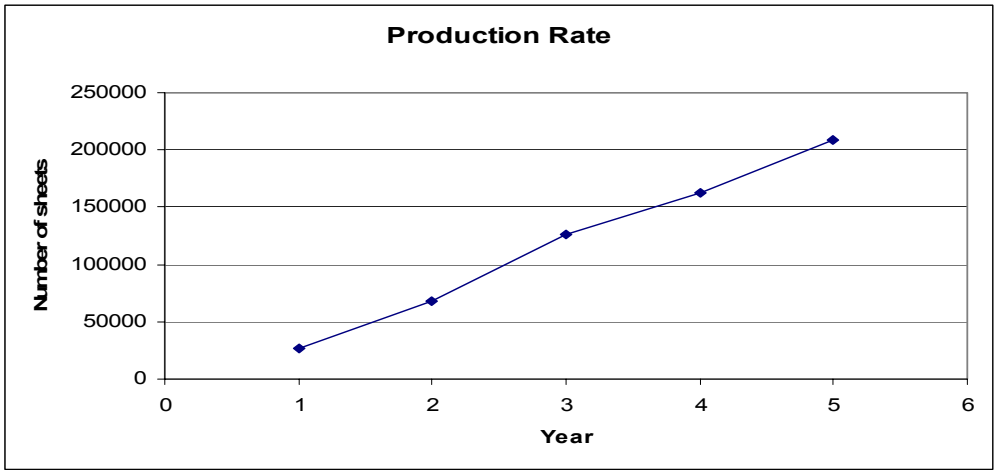


Figure 18: Production Growth Rate for 10 years

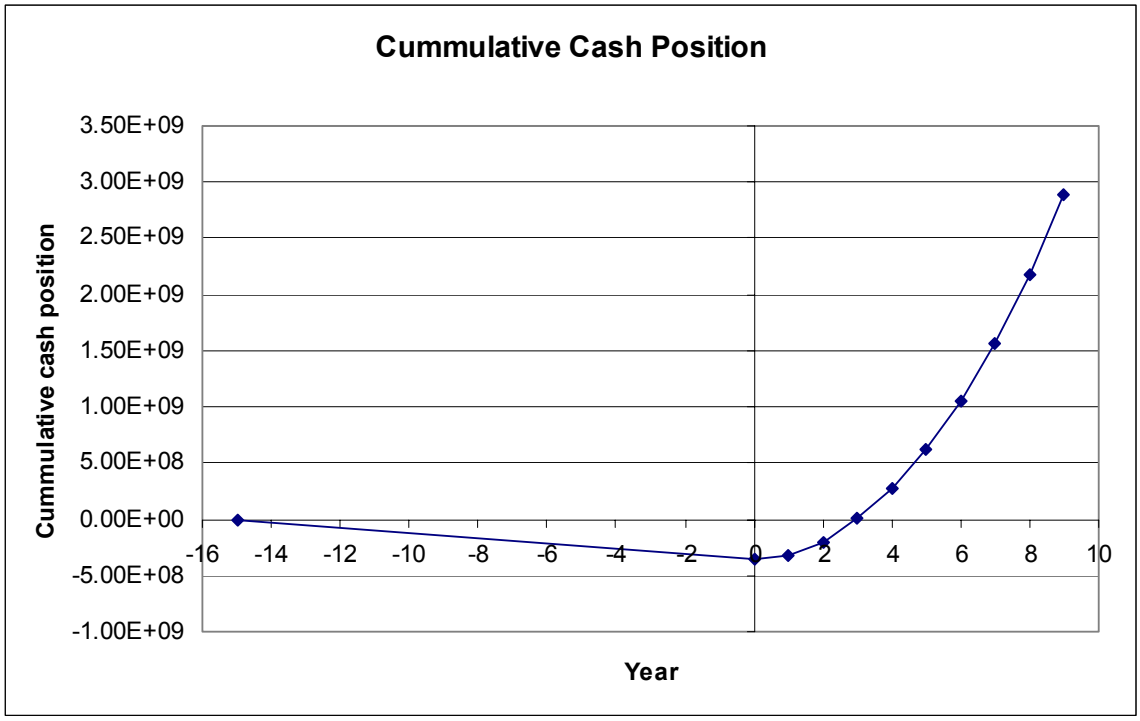


Figure 17: Cumulative Cash Position

As discussed above in the model, FCI will be recovered in three years after production. The marketing cost is increased by 20 % and the number of employees was increased by 25% to meet the need for the production growth.

Appendix

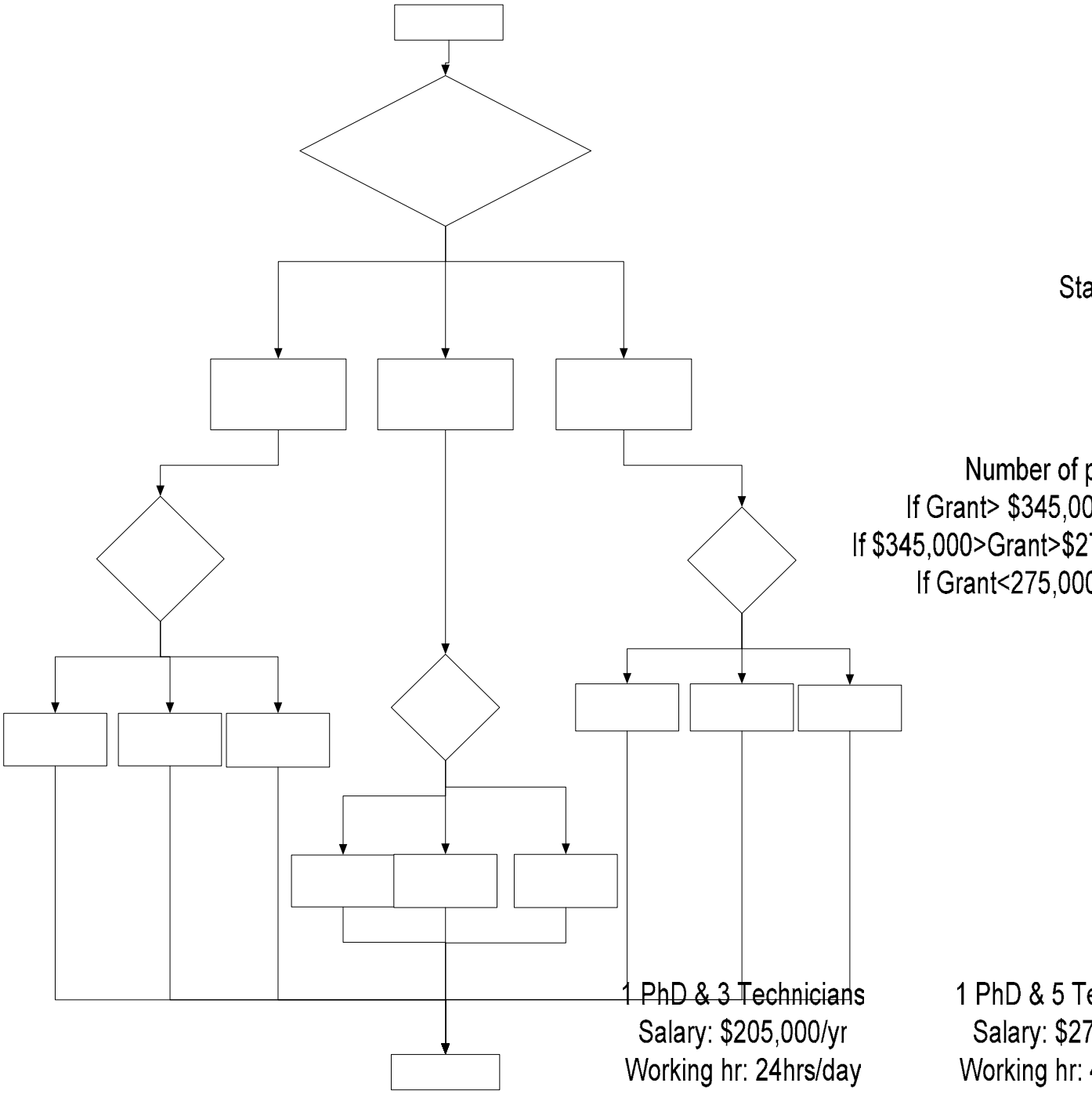
Break-even point calculations

Yr		Rate of production (sheet/yr)	% Market share	Revenue/yr	Staff (Increase in staff 5%)	Raw material cost	Marketing	Total product cost/yr
1		72000	0.0097919	43200000	1000000	14076000	602340	85678535.5
2		93600	0.0114384	56161872	1100000	18664776	572223	90337198.4
3		121680	0.0133317	73012867	1210000	24749492.98	543611.85	96503308.2
4		170352	0.0166989	1.02E+08	1270500	35342275.97	516431.26	107129415
5		238492.8	0.0208764	1.43E+08	1334025	50468770.08	490609.69	122293616

Total market demand

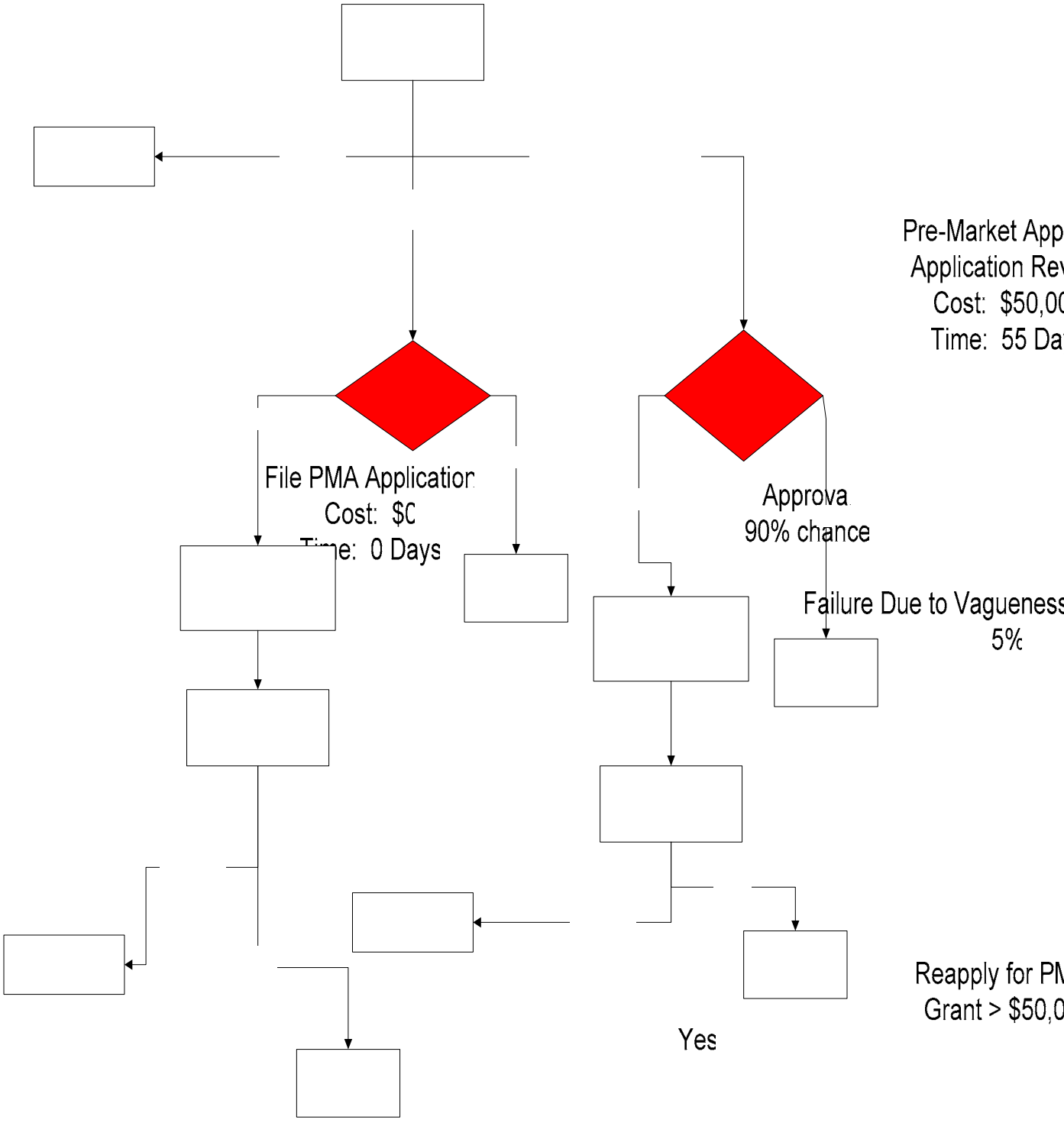
Yr	Sale price with 2% inflation rate	Req'd sheet for Burn/yr (1% growth)	Req'd sheet for Wound/yr (1.2% growth)	Req'd sheet for Ulcers/yr (14% growth)	Total production demand in the market
		680000	725000	2350000	
1	600	736342	797594	5818513	7353049
2	600.02	743705.42	805569.94	6633104.82	8182980.2
3	600.04	751142.4742	813625.6394	7561739.495	9127107.648
4	600.06	758653.8989	821761.8958	8620383.024	10201398.88
5	600.08	766240.4379	829979.5148	9827236.647	11424056.68

Appendix A



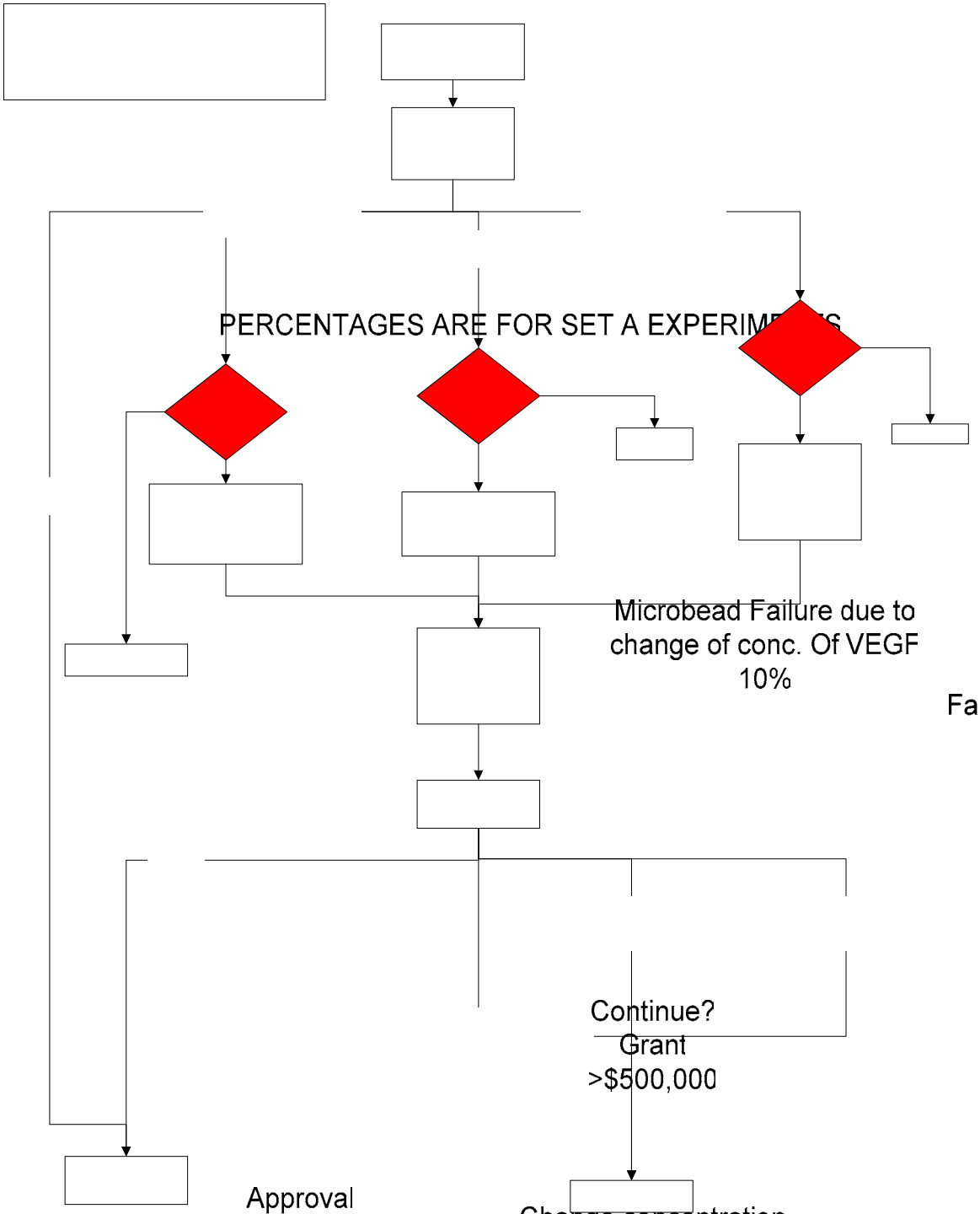
First Stage Variables: Decisions Made Prior to FDA Approval Process

Number of
experiments rur

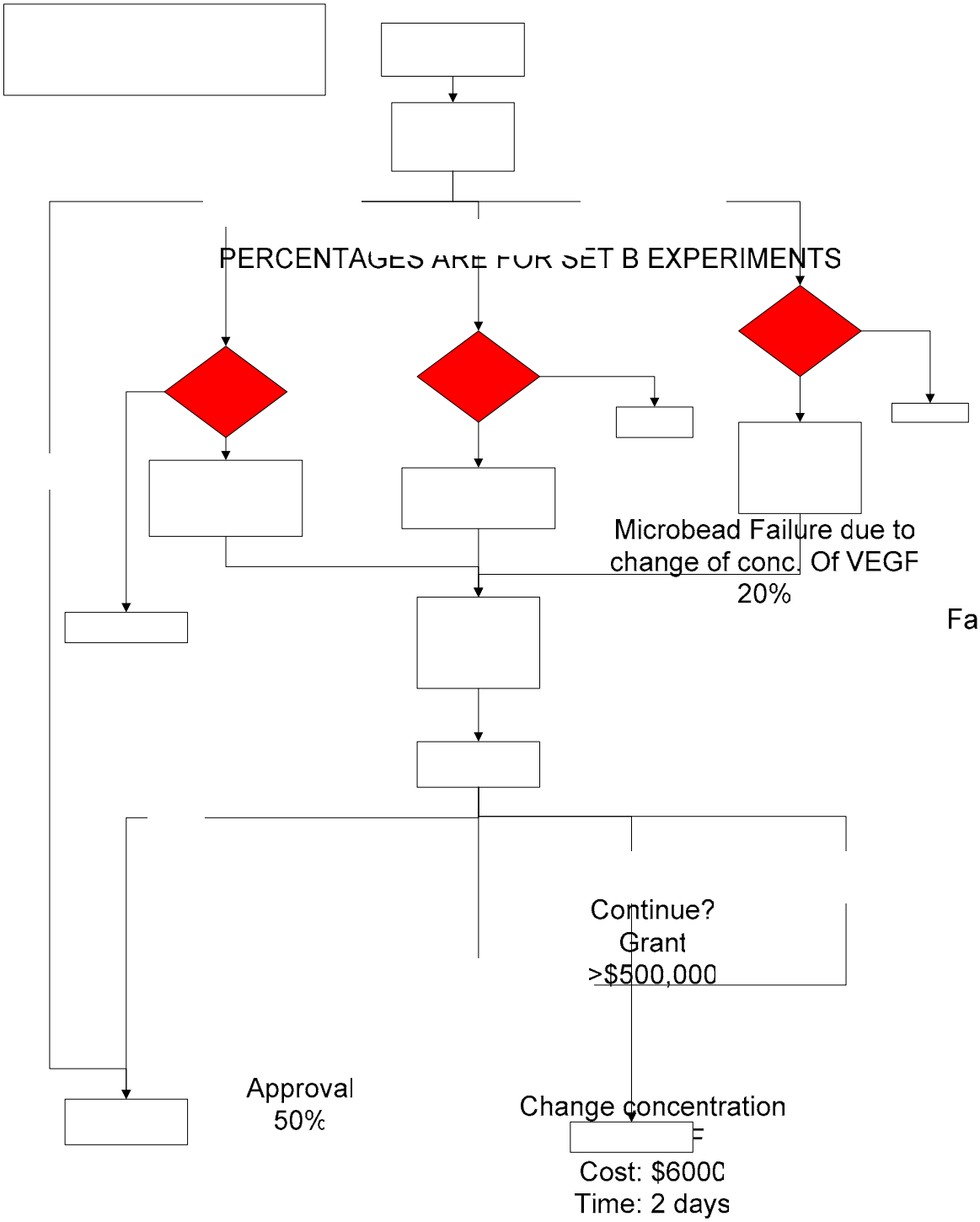


Pre-Market Approval Decision Tree: All Percentages Remain the Same for All Personnel Decisions and Experiment Sets

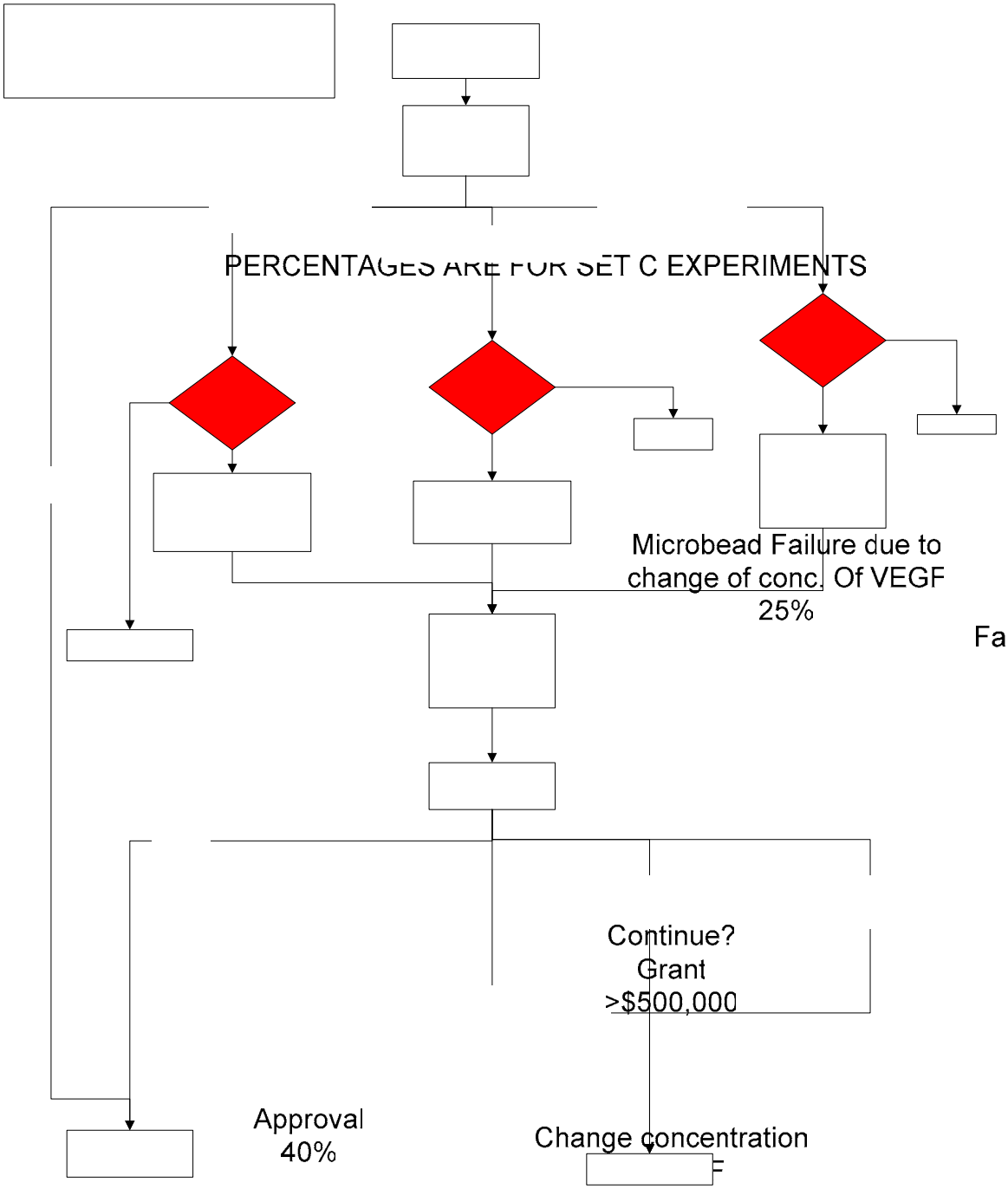
Re-Apply for Pre-Market Approval Review
Cost: $50,000



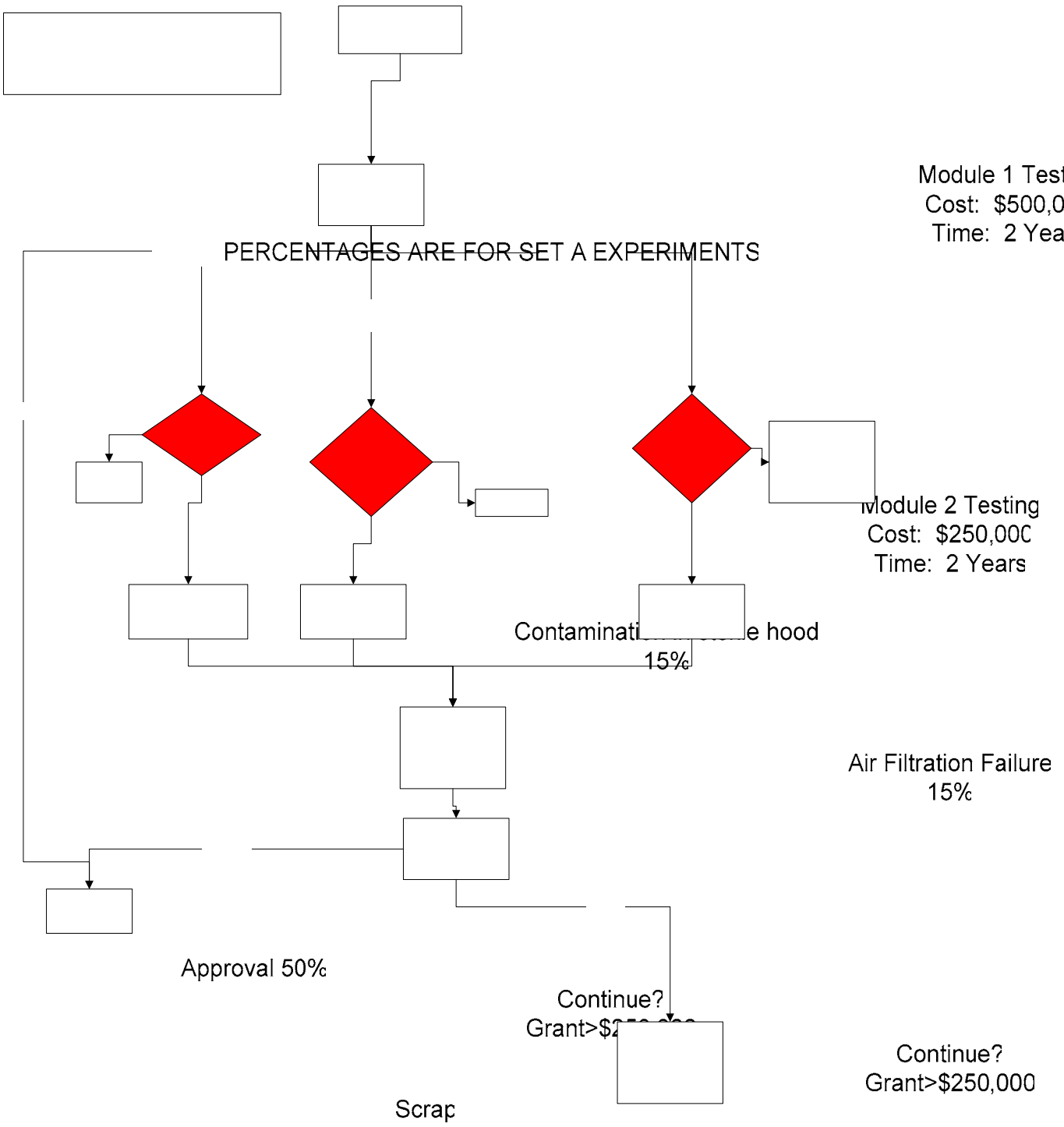
Module 1 Decision Tree: Percentages are for Experimental Set A



Module 1 Decision Tree: Percentages are for Experimental Set B



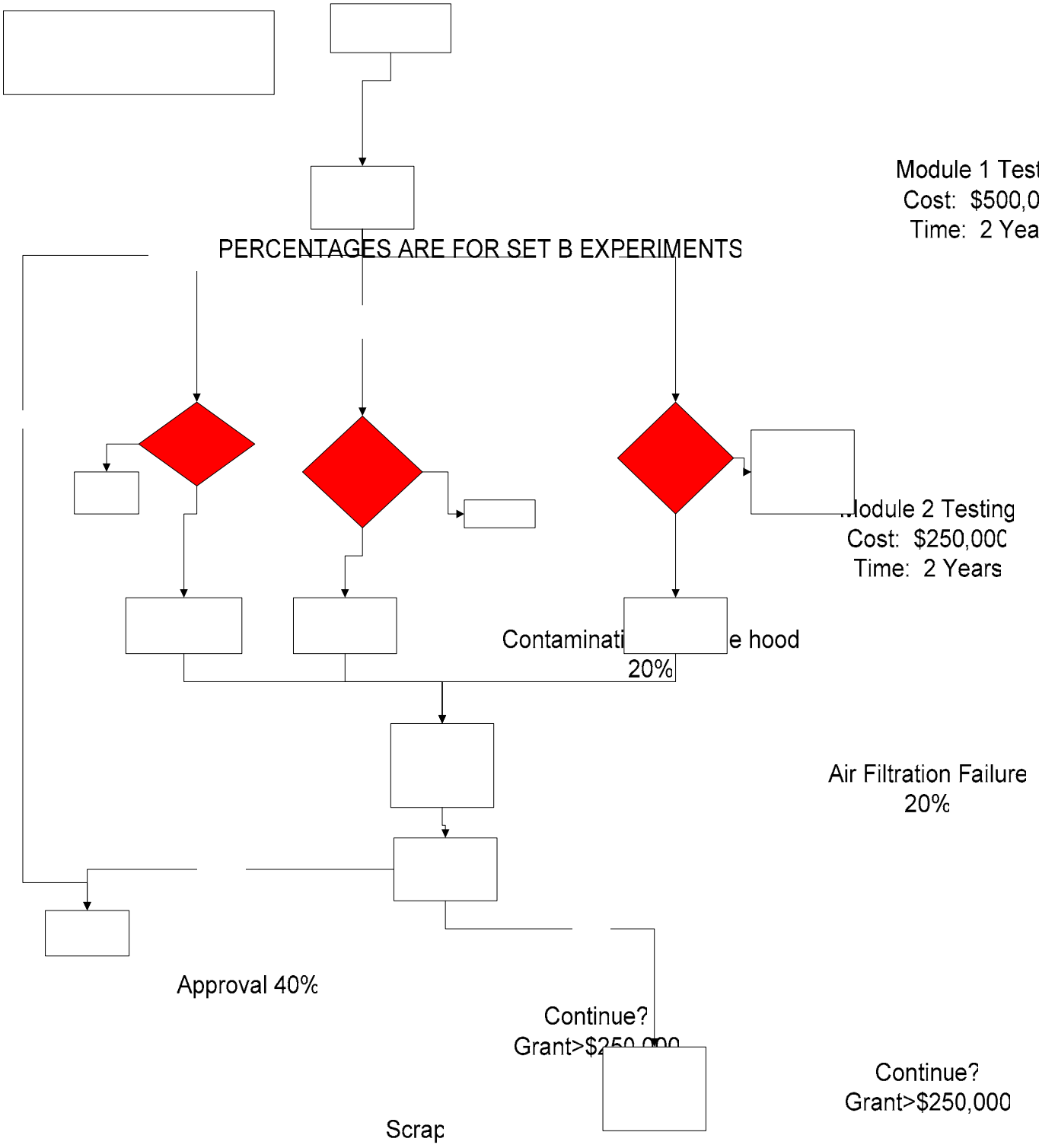
Module 1 Decision Tree: Percentages are for Experimental Set C



Module 2 Decision Tree: Percentages are for Experimental Set A.

Purchase new hood
Cost: $ 50,000
Time: 7 days

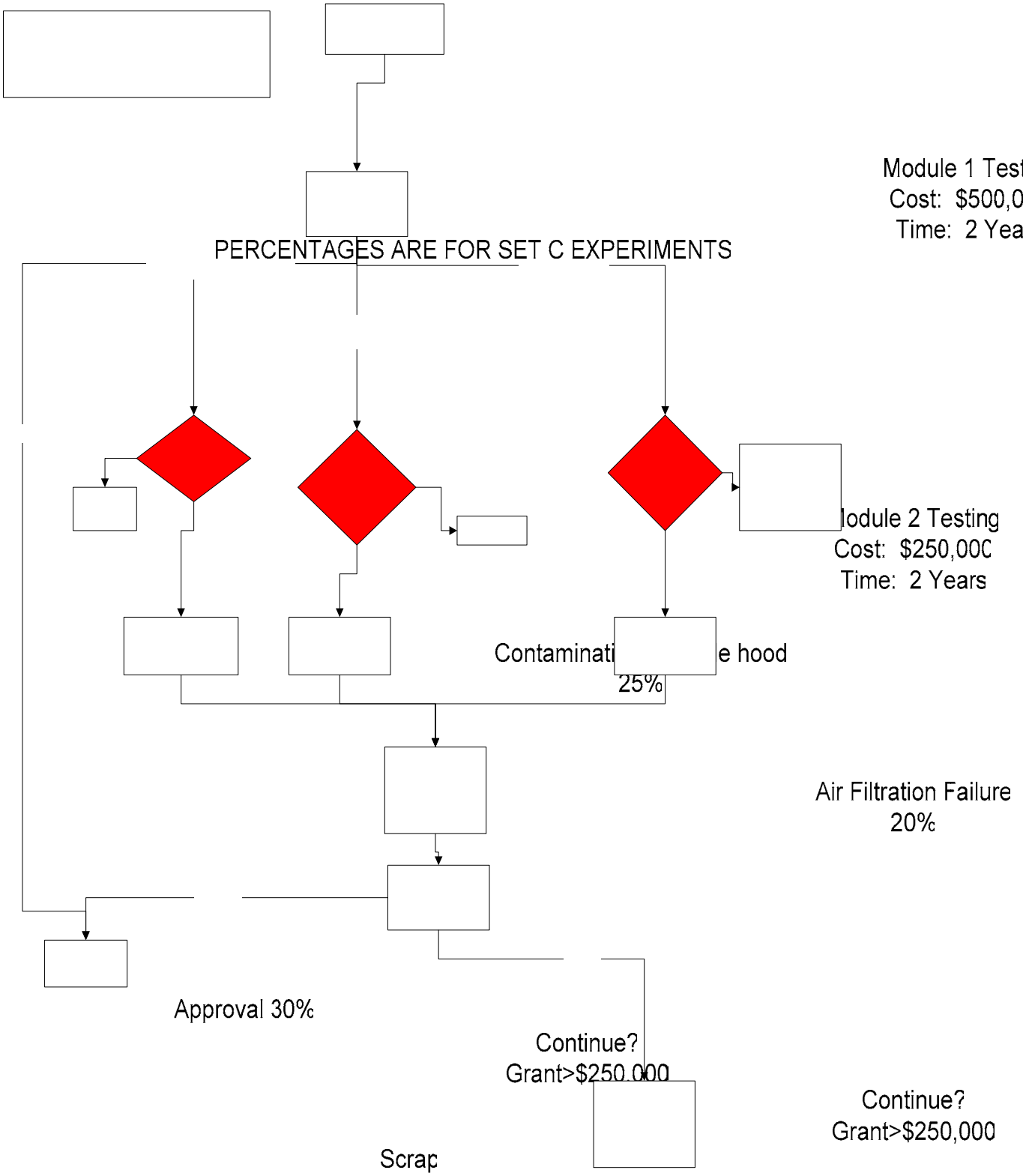
Replace Air
Filtration System
Cost: $75,000
Time: 14 days



Module 2 Decision Tree: Percentages are for Experimental Set B

Purchase new hood
Cost: $ 50,000
Time: 7 days

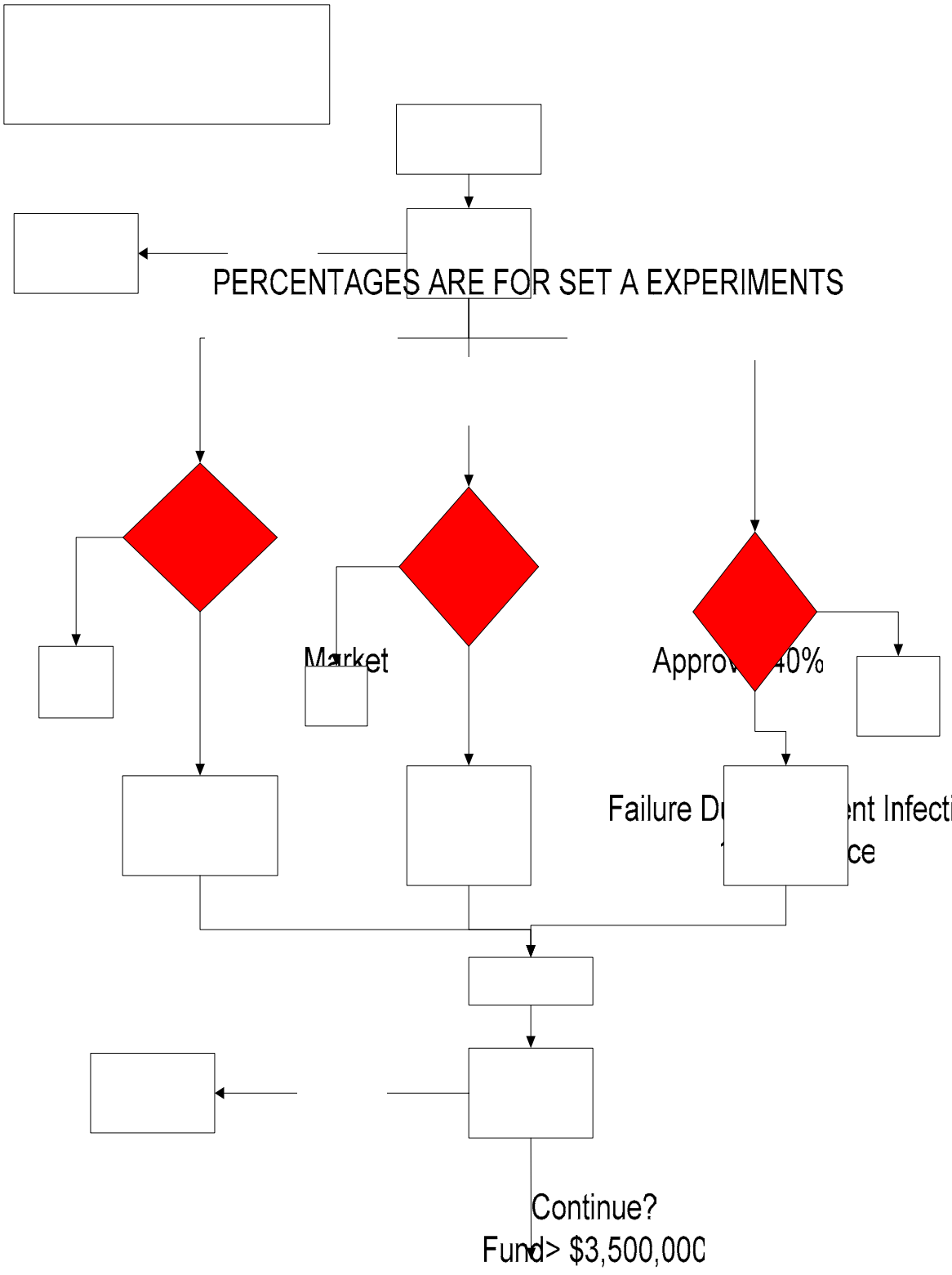
Replace Air Filtration System
Cost: $75,000
Time: 14 days



Module 2 Decision Tree: The Percentages are for Experimental Set C

Purchase new hood
Cost: $ 50,000

Replace Air
Filtration System

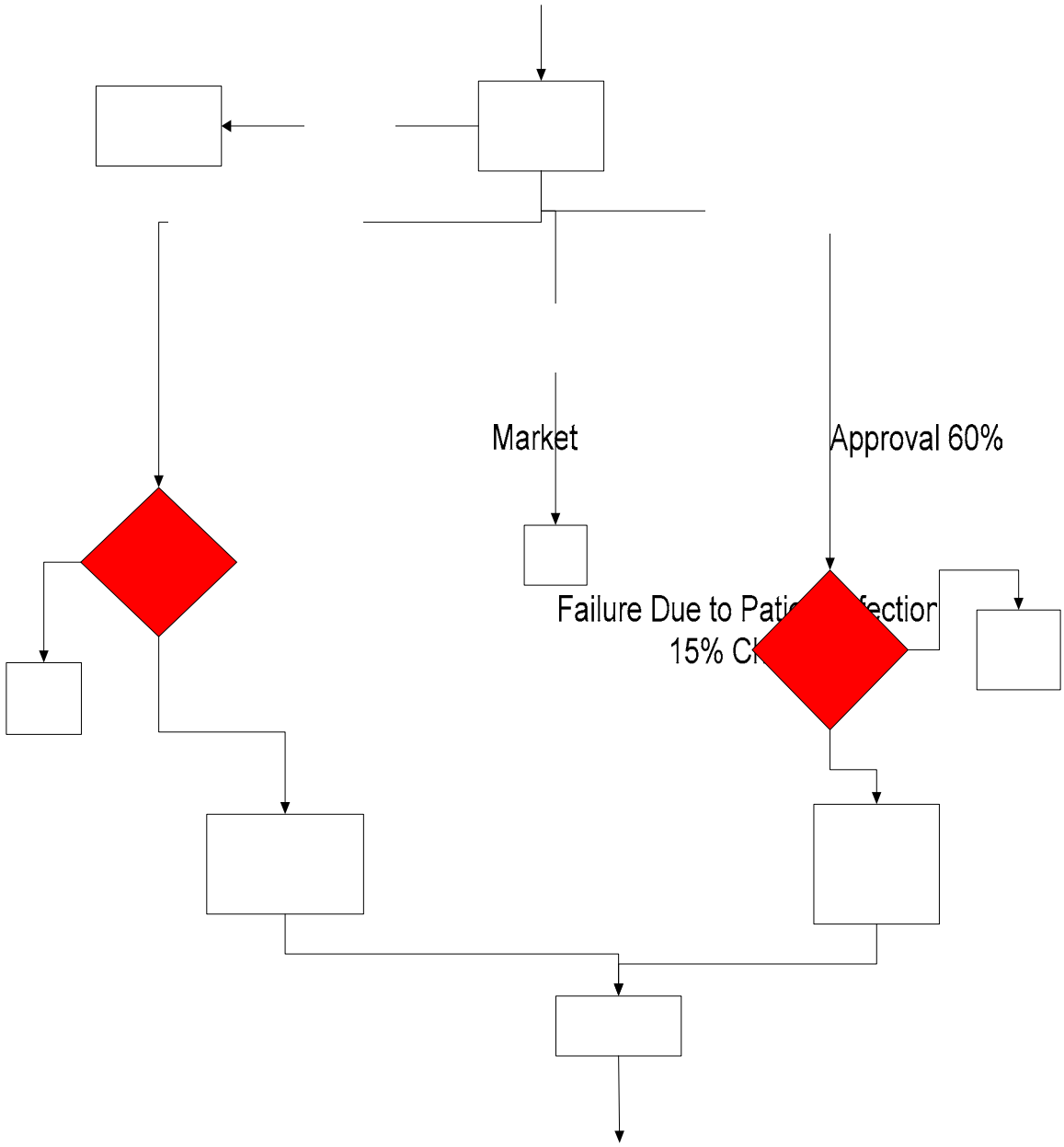


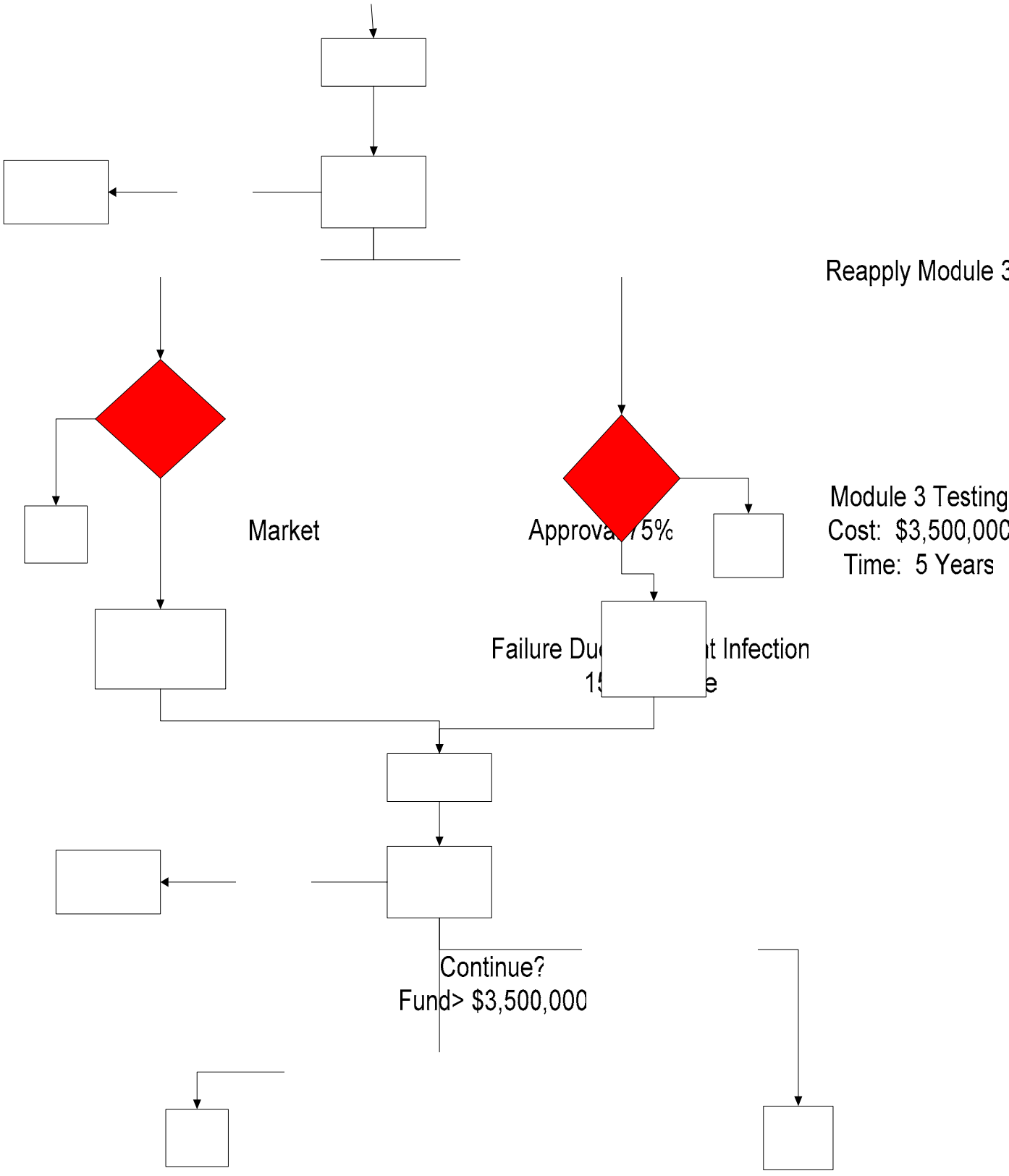
Module 2
Cost: $2
Time: 2

Module 3
Cost: $3,
Time: 5

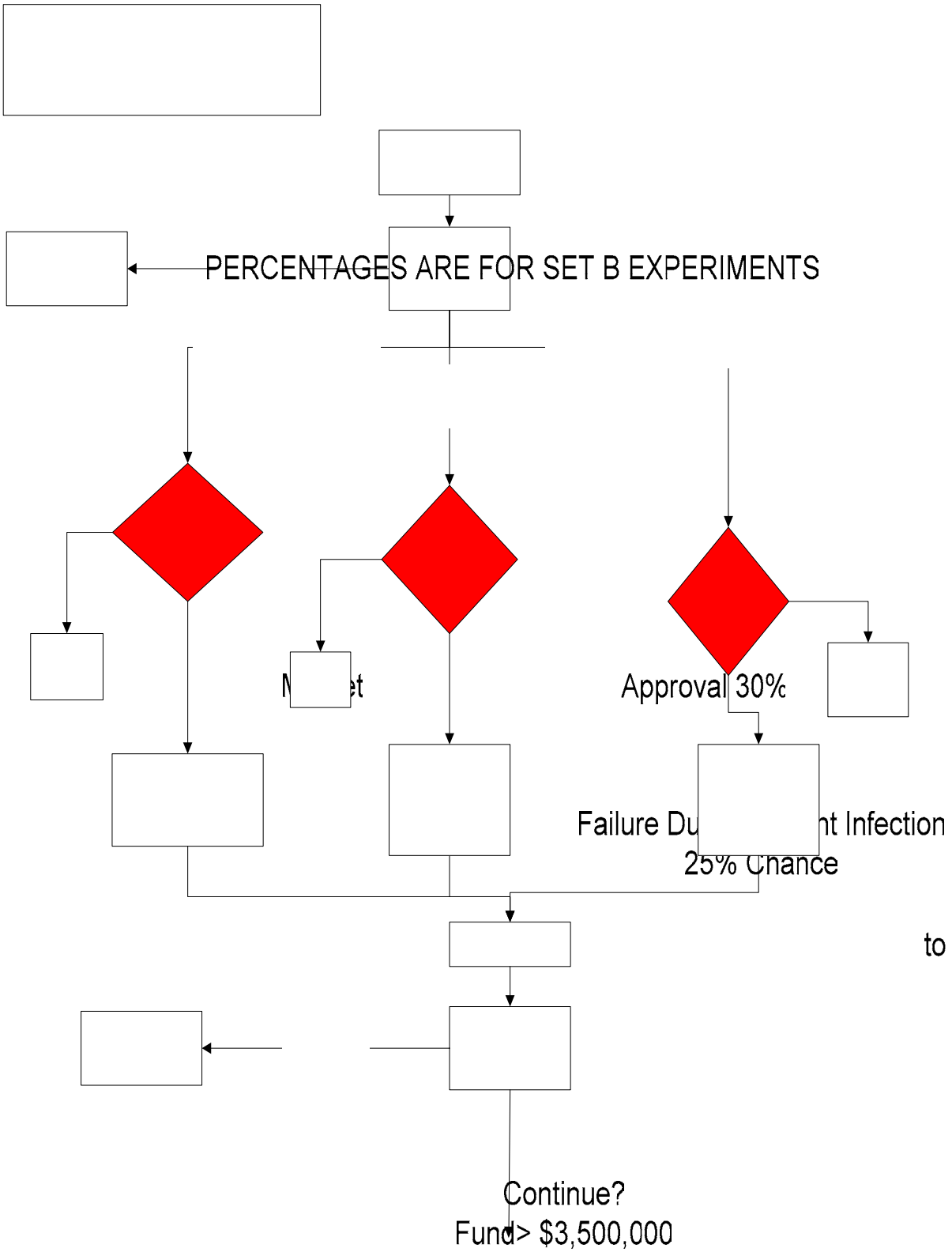
Failure
to immune sys
15

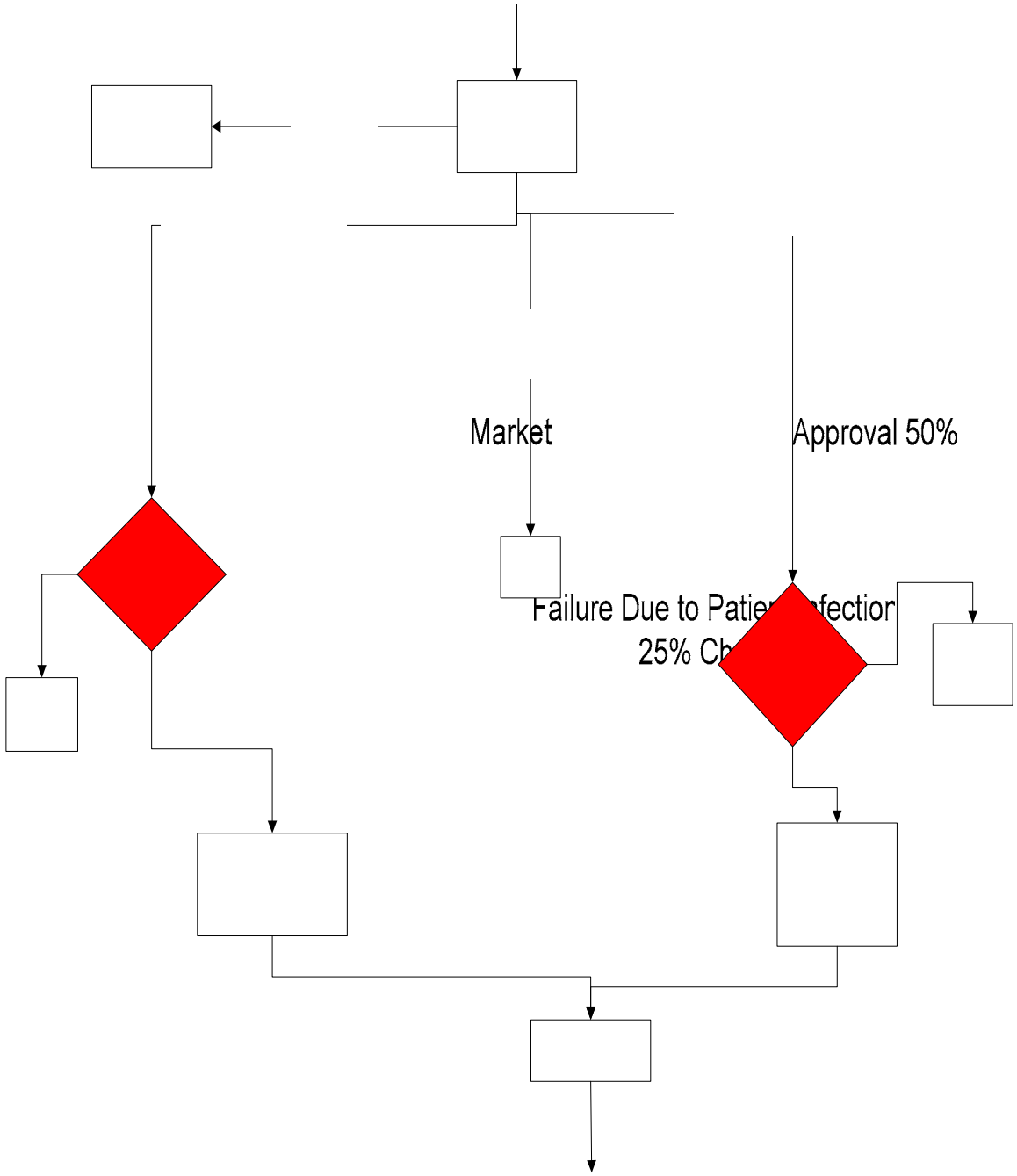
Contin
Fund>$3,

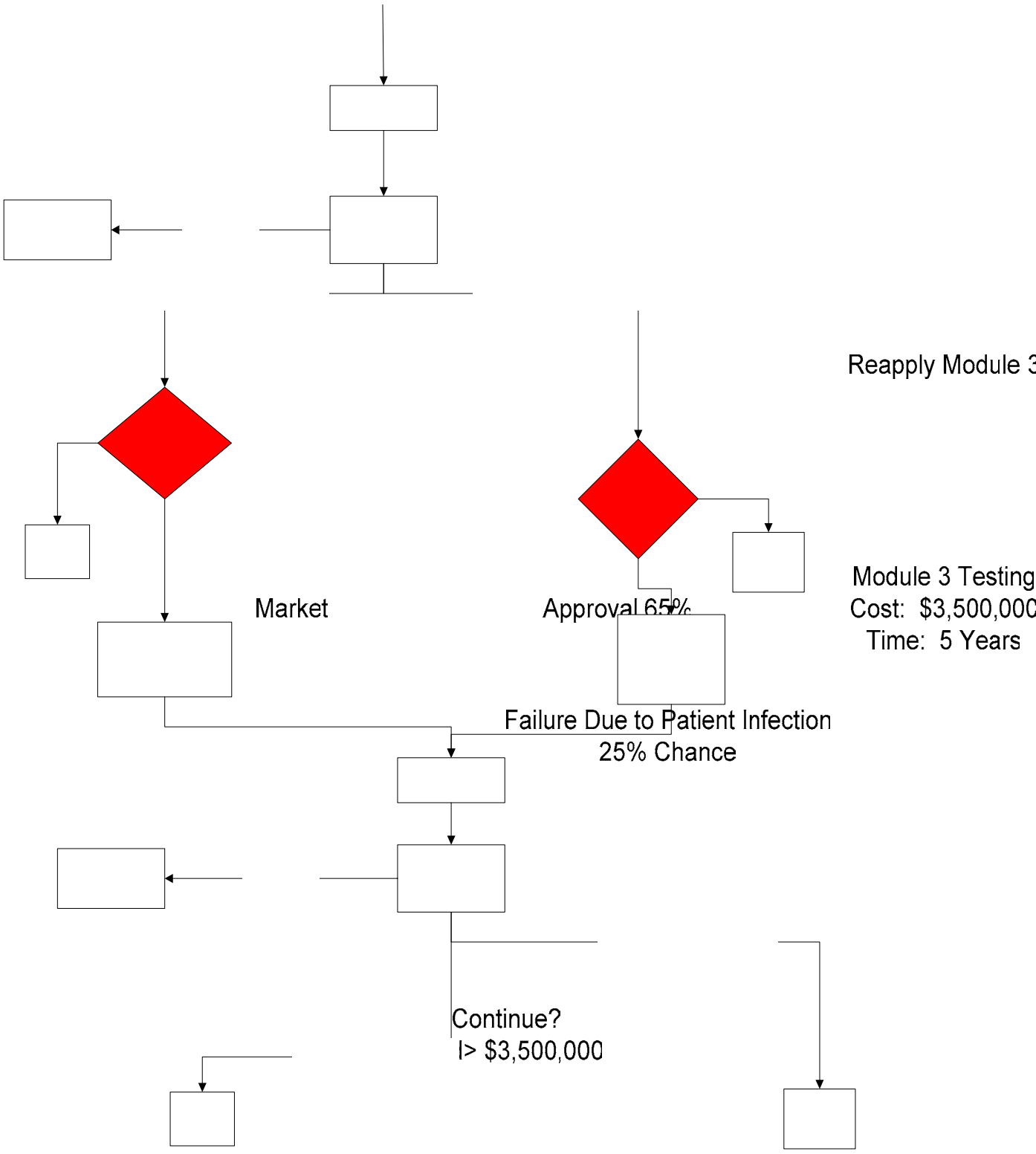

Market
Approval 60%
Module
Cost: $
Time:
Failure Due to Pati ection
15% C
Fa
to immune
Continue?
Fund> $3,500,000
Scrap



Module 3 Decision Tree: Percentages are for Experimental Set A

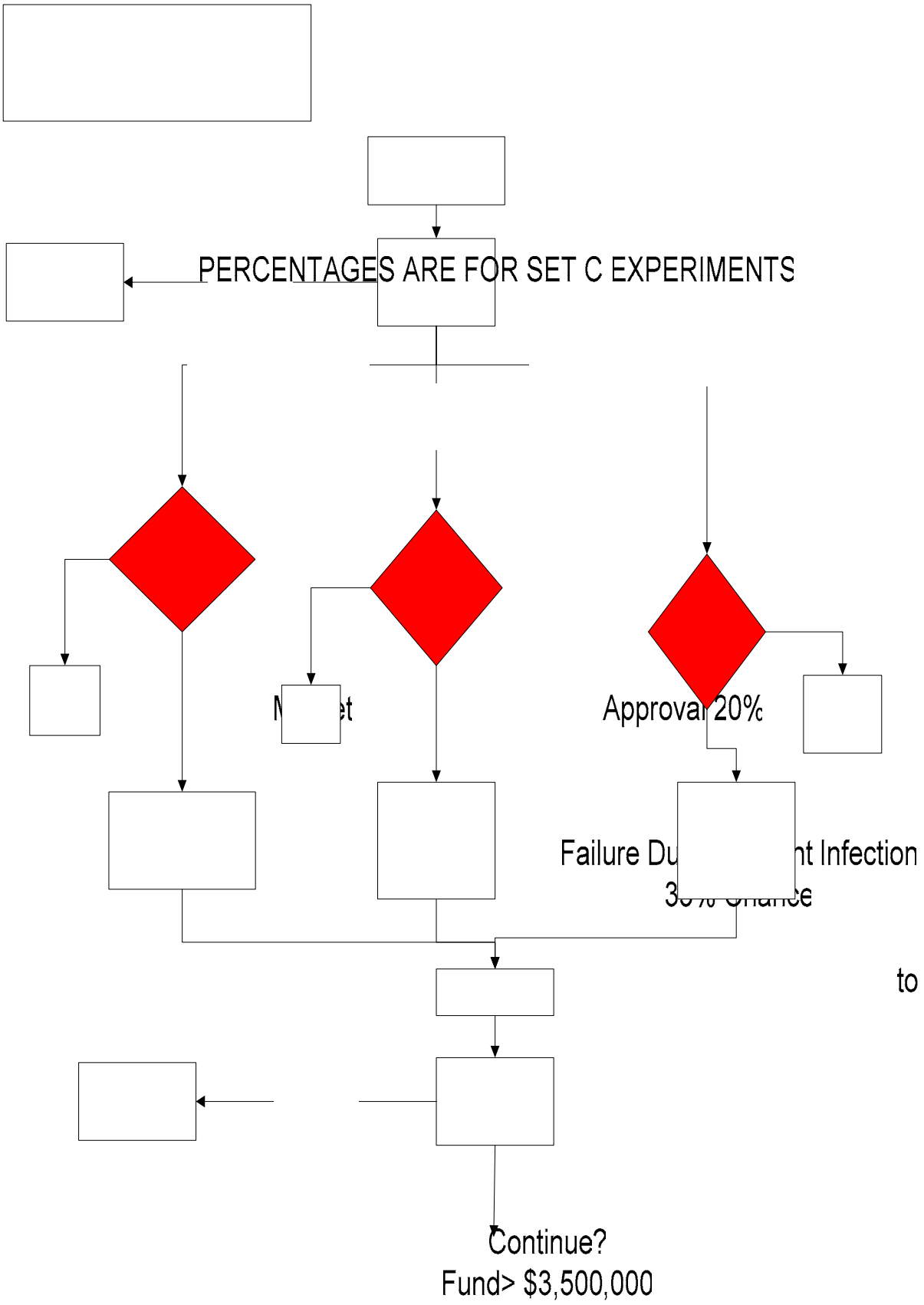

PERCENTAGES ARE FOR SET B EXPERIMENTS
Approval 30%
Failure Du nt Infection
25% Chance
Continue?
Fund> $3,500,000
Module 2
Cost: $2
Time: 2
Module 3
Cost: $3,
Time: 5
Failure
to immune syst
15%
Contin
Fund>$3,
Scrap
Scrap

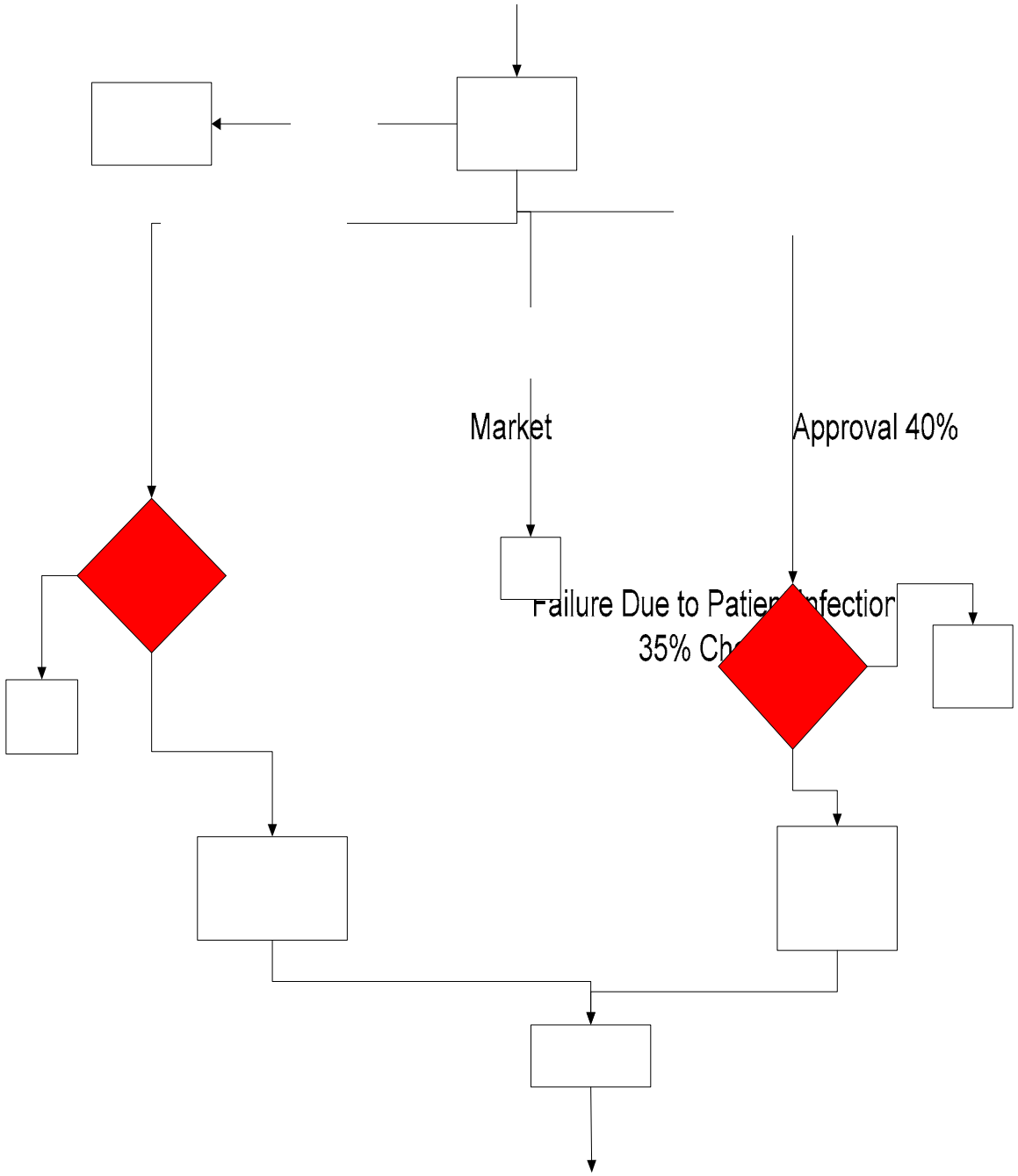

Market
Approval 50%
Module
Cost: $
Time:
Failure Due to Patie fection
25% Ch
Fa
to immune
Continue?
Fund> $3,500,000
Scrap

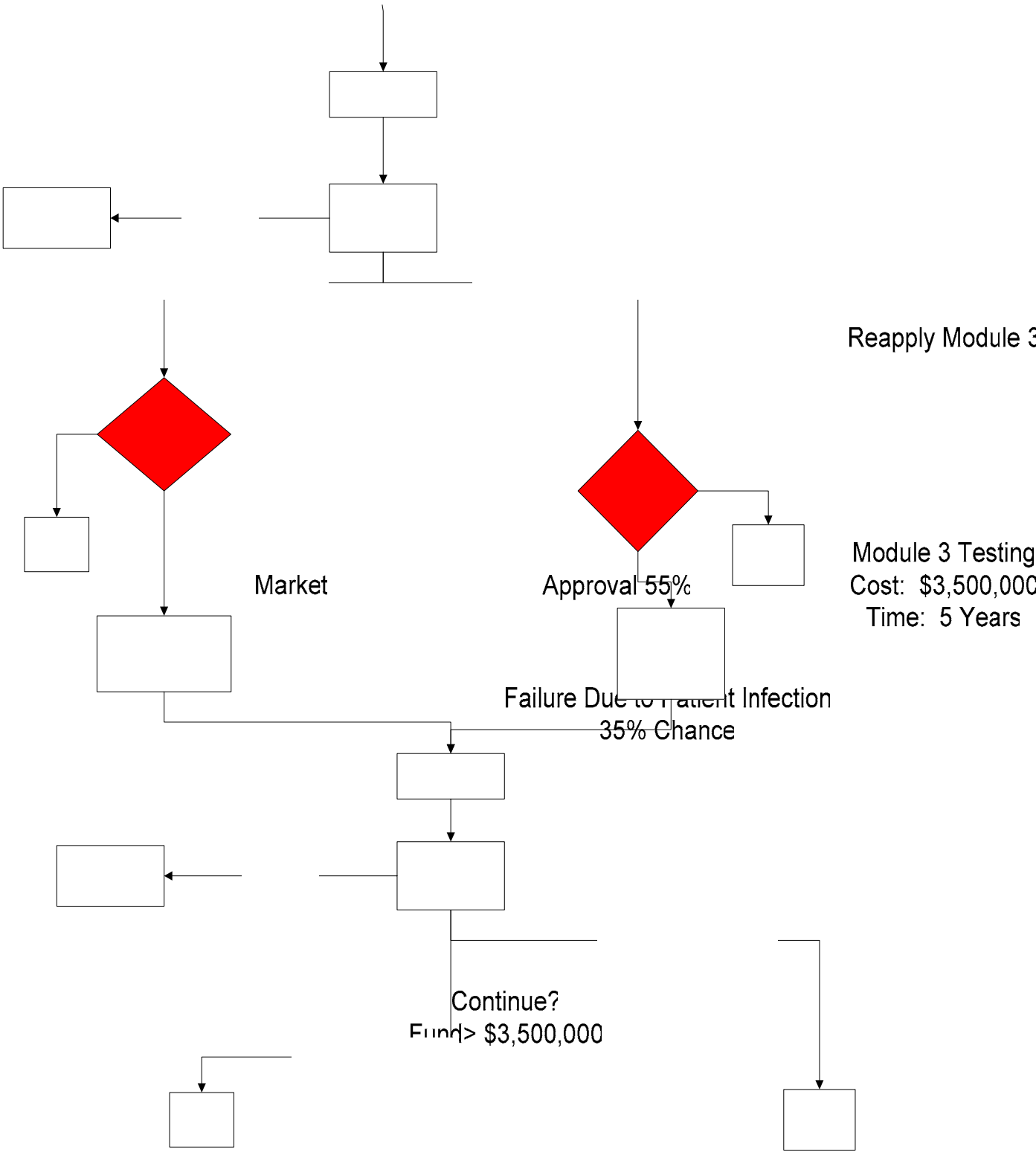


Module 3 Decision Tree: Percentages are for Experimental Set B

Add Antibiotics into the


PERCENTAGES ARE FOR SET C EXPERIMENTS
Approval 20%
Continue?
Fund> $3,500,000
Module 2
Cost: $2
Time: 2
Module 3
Cost: $3,
Time: 5
Failure
to immune syst
15%
Contin
Fund>$3,


Market
Approval 40%
Module
Cost: $
Time:
Failure Due to Patien fection
35% Ch
Fa
to immune
Continue?
Fund> $3,500,000
Scrap

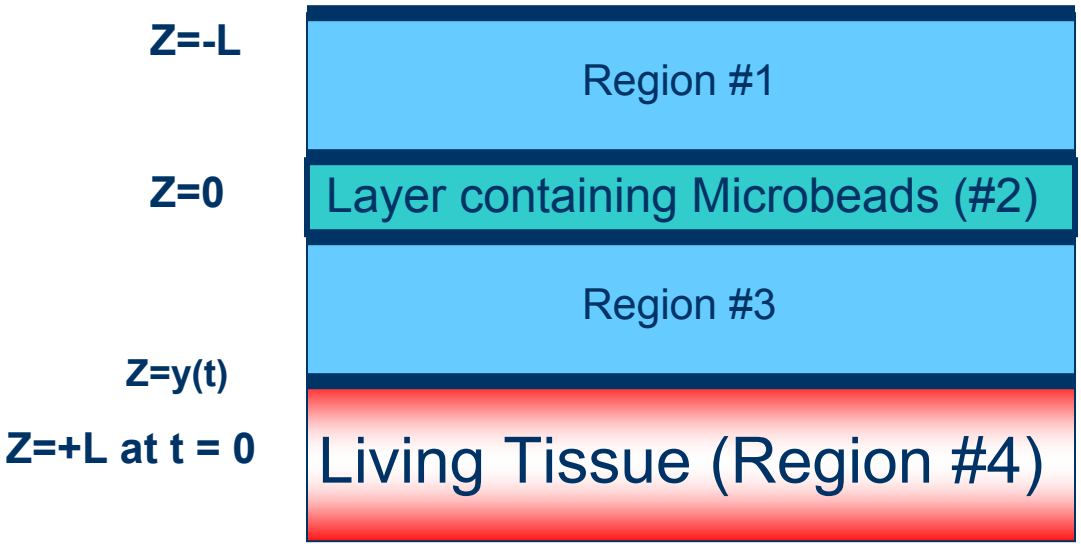


Module 3 Decision Tree: The Percentages are for Experimental Set C

Diffusion Model

The diffusion model presented below was created to estimate the concentration gradient of VEGF in the dermal replacement graft based on the concentration and placement of the microbeads in the graft. It should be noted that unlike our final product, which has microbeads placed on the top layer of the graft, this model assumes that the microbeads are in the center of the graft. The model may be modified to fit any location for the placement of the microbeads, and the model below is given only as an example to show its predictive capability.

Model Layout



In the above model, there is no flux across the top layer out of region #1, so it can be said that:

$$\frac{\partial c_1}{\partial z} = 0$$

The mass balance across each layer serves to provide other boundary conditions. The middle layer (Region #2) releases VEGF with a concentration c* at a rate r*. The rate of release (r*) is evaluated initially as a function of time, but it will be later shown to represent a constant rate of release.

For region #1, assume a solution of the following form:

$$c_1 = K_1 + K_2 erf(\eta)$$

where:

$$\eta = \frac{-z}{t\sqrt{\alpha_2}}$$

Applying the boundary condition @ z = +L:

$$\frac{\partial c_1}{\partial z} = 0$$

We find that K_2 must equal zero. We find that:

$$c_1 = K(t), c_1 \neq f(z)$$

Similarly for region #3:

$$c_1 = K_3 + K_4 erf(\theta)$$

where:

$$\theta = \frac{z}{\alpha_3\sqrt{t}}$$

The rate of release is given by:

$$r^*(t) = D_1 \frac{\partial c_1}{\partial z} + D_1 \frac{\partial c_3}{\partial z}$$

where the derivatives are evaluated at z = 0. Continuing with calculations:

$$r^*(t) = \frac{D_1 K_4(t)}{\alpha\sqrt{t}}$$

Thus:

$$K_4(t) = \frac{r^*(t)\alpha_3\sqrt{t}}{D_1}$$

From the previous expression, we can conclude:

$$c_1 = c_3 = \frac{\alpha_3 r^*(t)\sqrt{t}}{D_1} erf(\eta) + K_3(t)$$

At z = y(t), the diffusive flux out of the graft is carried away by the blood stream. This is represented with the following rate expression:

$$D_1 \frac{\partial c_3}{\partial z} = kc_3$$

Substituting and canceling terms at z = y(t):

$$\sqrt{t}\alpha_3 r^*(t) \frac{\partial}{\partial z}[erf(\theta)] = kc_3$$

$$\frac{\partial}{\partial z}[erf(\theta)] = \frac{1}{\alpha_3\sqrt{t}} e^{-y^2/\alpha_3\sqrt{t}}$$

$$r^*(t) e^{-y^2/\alpha_3\sqrt{t}} = k\frac{\alpha_3\sqrt{t} r^*(t)}{D_1} erf\left(\frac{y(t)}{\alpha_3\sqrt{t}}\right) + kK_3(t)$$

Therefore, $K_3(t)$ is equal to the following expression:

$$K_3(t) = \frac{r^*(t)}{k} e^{-y^2/\alpha_3\sqrt{t}} - \frac{\alpha_3 r^*(t)\sqrt{t}}{D_1} erf\left(\frac{y}{\alpha_3\sqrt{t}}\right)$$

Our rate of vascularization is defined to be equal to $k_g c_3$. Therefore:

$$\frac{dy}{dt} = k_g \frac{\alpha_3\sqrt{t} r^*(t)}{D_1} erf\left(\frac{y}{\alpha_3\sqrt{t}}\right) + K_3(t)k_g$$

Substituting in the determined expression for $K_3(t)$ in the previous expression, we find:

$$\frac{dy}{dt} = \frac{k_g r^*(t)}{k} e^{-y^2/\alpha\sqrt{t}}$$

We have defined r*(t) to be the release rate of VEGF into the graft. Because a steady-state release rate is reached very quickly, r*(t) = r* = a constant term.

$$\frac{dy}{dt} = \frac{k_g r^*}{k} e^{-y^2 / \alpha_3 \sqrt{t}}$$

Integrating the previous expression, we find that:

$$y(t) = \frac{k_g r^*}{k}\left[\left(e^{-y^2/\alpha\sqrt{t}}\left(t - \left(\frac{\sqrt{t}(y(t))^2}{\alpha_3^2}\right)\right)\right) - \left(\frac{(y(t))^2}{\alpha_3^2}\int_{-y^2/\alpha\sqrt{t}}^{\infty}\frac{e^{-t}}{t}dt\right)\right] + C$$

This expression may be used to predict the concentration of VEGF at any point and for any time in the graft. Because the previous expression is tedious, it should be noted that the differential expression that was integrated to obtain the result can also be evaluated numerically to obtain a fairly accurate solution.

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[1] Transplant Rejection Information: http://www.ghltp.org/education/transplant_facts.html

[2] Integra Dermal Regeneration Template Multi Center Clinical Trials <http://www.integra-ls.com/bus-lifesci_main.htm> accessed 3/12/2004

[3] Integra Dermal Regeneration Template Product Description <http://www.integra-ls.com/bus-lifesci_main.htm> accessed 3/12/2004

[4] King, Timothy W.; Patrick, Charles W., Jr. Development and in vitro characterization of vascular endothelial growth factor (VEGF)-loaded poly(DL-lactic-co-glycolic acid)/polyethylene glycol microspheres using a solid encapsulation/single emulsion/solvent extraction technique. Laboratory of Reparative Biology and Bioengineering, Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA. Journal of Biomedical Materials Research (2000), 51(3)

[5] Cao X., Schoichet MS. Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders. Biomaterials 1999; 20: 329-339.

[6] William L. Murphy, Martin C. Peters, David H. Kohn, David J. Mooney. Sustained release of vascular endothelial growth factor from mineralized poly(lactde-co-glycolide) scaffolds for tissue engineering. Biomaterials. (2002), 21: 2521-2527.

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[8] Yannas, I. V.; Lee, E.; Orgill, D. P.; Skrabut, E. M.; Murphy, G. F. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Fibers Polym.

Lab., Massachusetts Inst. Technol., Cambridge, MA, USA. Proceedings of the National Academy of Sciences of the United States of America (1989), 86(3),