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Highly controlled collagen nanoloom

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Highly Controlled Collagen Nanoloom

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Technical Design Report

Highly Controlled Collagen Nanoloom Project 6

Final Report

Sponsor: National Science Foundation Design Advisor: Professor Jeffrey Ruberti

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December 8th, 2005

Department of Mechanical, Industrial and Manufacturing Engineering College of Engineering, Northeastern University Boston, MA 02115 Artificial tissue based on collagen is gaining wider use in the medical community for tissue replacement. Current collagen assembly methods only allow for the production of disorganized, weak scaffolds that can only be used in limited applications such as skin grafts. There is a greater need to create highly organized, load-bearing collagen matrices such as ligaments and corneas. It is essential that a Collagen Nanoloom be developed in order to further advance artificial tissue production. The goal of the Collagen Nanoloom device is to replicate the environments of collagen producing cells known as fibroblasts. The Nanoloom will consist of two thermally different environments, one at 4°C and the other and 37°C, controlled by Peltier coolers and Thermofoil heaters, respectively. Collagen solution will be pumped through the "cool" side and allowed to go through a membrane with 60 nm perforations to the "warm" side. The theory is that the collagen fibers will go through the perforations and be caught by a substrate on the "warm" side allowing for manually constructed structures. To manually align the fibers, nano-motion control stages will be used to move in the x-y-z directions to keep the fibers in tension. A prototype of the Collagen Nanoloom will be constructed to test the theory of this technology.

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INTRODUCTION

According to the World Health Organization, around 10 million people in the world suffer from corneal blindness and only about 1% (100,000 people) receives corneal transplants each year. This small ratio is due to the short supply of donor corneas and unsuitability of some patients to receive a corneal graft [4].

The main goal of tissue engineers is to recreate the manufacturing facilities of the body and produce artificial tissue for the human body. The artificial tissue would be a suitable replacement for diseased or damaged tissue, tendons, cornea, and ligaments.

One of the major building blocks for these types of tissue is collagen fibers. Collagen is a fibrous protein that is found throughout the body and is the primary load bearing molecule. It can be found in the structure of skin, bones, tendons, ligaments, cornea, teeth, cartilage, and blood vessels. The artificial production of collagen fibers can lead to the development of artificial tissue and is the concentration of the collagen nanoloom report. The collagen nanoloom will be a step in producing highly aligned collagen fibers that can withstand in vivo loads.

PROBLEM STATEMENT

The main objective of this project is to design and develop a collagen nanoloom which is capable of "printing" artificial collagen fibrils that are highly aligned on the nanoscale with equal load bearing capabilities as biological tissue located in the body but concentrating on the corneal stroma. The ultimate goal of the project is to develop a nanoloom which is capable of printing in three dimensions, catering to the unique complex matrix structures that are located in different anisotropic tissue located throughout the body. However, for the purpose of proof of technology, the short term goal is to develop a nanoloom which can print in two dimensions with the capability to print in three dimensions. After a proof of technology phase has been completed, the collagen nanoloom can be tuned to be able to print such three dimensional anisotropic structures as those found in tendons, ligaments, and corneas, among others. The ultimate promise of this technology is to provide a more effective means of creating load bearing, highly organized replacement tissue.

BACKGROUND

Collagen Background

The word collagen comes from the Greek word "kola," which means "glue." This describes the unique property of collagen polymers as scaffolding for the human body. Collagen is the most abundant protein in the human body. It makes up 75% of the body's skin and can be found in cartilage, bone, and intervertebral disk annulus fibrosus, among other types of tissue with in the body. Collagen is not limited

to just one type either; the human body alone consists of 20 different types of collagen. The four main types found in the human body are Type I, Type II, Type III, and Type IV. Type I collagen forms tendons, ligaments, and bones. Type II forms more then 50% of the protein in cartilage. Arteries, the intestine, and the uterus are supported and strengthened by Type III and Type IV provides a filter for the blood capillaries and the glomeruli of the kidneys. Type IV also forms a basal lamina of epithelia. [10]

A collagen molecule (monomer) is a triple-helix structure that comprises of three tightly woven chains. The chains consist of three types of amino acids (Glycine, Proline, and Hydroxyproline). The tightly woven chains form the collagen fibrils, as seen in Figure 1. The collagen fibrils are typically arrayed in a highly-organized anisotropic manner and make up the majority of all tensile load-bearing tissue in the body. [9]



Figure 1: Diagram showing the structural hierarchy of Collagen Fibers [13]

Collagen is the primary load bearing connective tissue in animals. Such tissue includes the cornea, ligaments, tendons, etc [7, 8]. Within the body, collagen mainly experiences tensile loads. Collagen fibrils tend to be oriented in a way that in vivo (within the body) loads are transmitted along their long axis. The collagen fibrils assemble into high-density, well organized matrices, which are excellent for transmitting mechanical loads. Collagen molecules self-assemble to form a supramolecular material which consists of more than one collagen molecule. It is a process which other biological material, such as DNA, is acquired. The polymerization process that collagen specifically undergoes is called fibrillogenesis.

There are three main stages in the production of collagen when it is being produced by fibroblast cells. On the surface of the fibroblast cells, tube-like depressions called "crypts" or "fibropositors" extrude from the surface. Procollagen molecules are transported to these crypts where they are processed and assembled. Once the procollagen molecules are processed and assembled, they are secreted into the extra-

cellular space (the space outside the cells) in a vectorial discharge. Extra-cellular enzymes cut off the N and C-terminals of the discharged procollagen which then forms tropocollagen or simply collagen [8, 10].

Collagen Production Background

The desirable result of a "nanoloom" system is to maintain accurate control of the layering orientation process during collagen polymerization such that the polymer upholds its mechanical and chemical properties at final shape. [Patent App, 20050019488]

The desired structure of collagen is to imitate the structure of collagen polymers in the human body. Collagen structures need to be aligned in a pattern to maximize its mechanical strength. Collagen polymers prearranged at random are weak when exposed to loads.

There have been several attempts to generate a corneal construct by putting together cornea cells with unassembled collagen fibrils but these have had limitations due to the complexity of the stromal structure (non-cross linked fibrils that are stacked in an anterior posterior manner). The cornea performs three major functions: protection, refraction, and transmission. The ability to fully simulate the function of a native cornea today is limited due to the lack of technology.

Patents and Existing Systems/Methods

The spinning method for aligning collagen is intended to produce corneal stromas with the right thickness. Droplets of collagen are dripped onto a warm spinning disc "that produces a highly oriented nanostructure of one or more layers of polymer material by exposing the monomer solution to a shearing flow and while under shearing for influence it utilizes the monomers' characteristic of self-assembling to form well oriented polymers" [Patent App, 20050019488]. "The alignment and position of the layering polymer nanostructure is controlled by the rate of flow of the processing material and relative velocity between the "delivery system" surface and the substrate. The rates are adjustable to control and match different polymer structure or "film" properties" [Patent App, 20050019488].

While the platform is spinning, the substrate is placed on the rotating platform which is enclosed in the temperature controlled system (See Figure 2). The substrate is pre-wetted and then the monomeric collagen solution can be dripped onto the spinning disc to form a layer of polymer solution that sticks onto the substrate. The centripetal force of the spinning motion aligns the collagen fibrils and many layers can be stacked on top by repeating these steps. The shearing process, however, does not allow enough flexibility for different fiber sizes and shapes. The system does provide a well oriented thin layered structure but also needs a unidirectional flow to construct different fibril structures.



Figure 2: Spinning apparatus that generates layer by layer oriented structure. (Patent Application 20050019488)

Bovine Collagen

Bovine collagen, which will be used for the nanoloom, is in a monomer form which can be obtained from tissue. It is a type I and III collagen monomeric solution; the monomer will self-assemble to form collagen polymers when exposed to specific set temperature and pH environment. The end result will be a gel-like bonding material that can not be rearranged easily. This inability to effectively rearrange the monomers after self-assembling has been a limiting factor in artificial tissue production.

MARKET ANALYSIS

Potential Uses for Nanoloom

Collagen is a vital component of load bearing tissue throughout the body. Humans and animals alike depend on this tissue to carry out their everyday activities but there is a problem when this connective tissue gets permanently damaged. The current methods for tendon and ligament repair all have certain drawbacks to them. The three methods of tissue repair are autographs, allograph, and xenographs.

Autographs are the best current option for tissue repair because there is a relatively high success rate for tissue transplants. The autograph process involves removing portions of tendon or ligament from a healthy section of the patient's body and transplanting them to the damaged area. Since it is a transplant between two parts of the same patient, there is little chance of rejection by the repaired area. Furthermore, there is little chance of infection due to the minimized time in between harvesting and transplantation of the ligament. There are some disadvantages of this process however. Removing ligament or tendon from a healthy part of the body weakens this part. This increases the chance of injury to the specific part of the body where harvesting occurred.

Allograph is considered to be the second choice for tendon and ligament repair. The allograph process involves removing tissue from another body, such as a deceased organ donor, and transplanting the tissue to the location needing repair on the patient's body. The benefit of this procedure is that the repair tissue is harvested from an outside body source instead of the patient's own body but the drawback is that there is an increased rate of rejection by the patients during the transplant. A transplant is not guaranteed to be successful even if a donor with the exact tendon is located and there is also an associated increase in infection resulting from a longer harvest-to-transplant time.

Xenographs are the last and least used ligament and tendon repair procedure. The process involves removing ligament or tendon from an animal specimen. The advantage to this process is the availability of different species. The disadvantages are that these heavily increase the risk of both rejection and infection in the patient. History has shown that there xenographs have a low success rate and are the least practiced method.

Given the disadvantages of the current tissue repair procedures, the option of the collagen nanoloom will be discussed. The advantage is the production of highly aligned collagen fibrils with a generic matrix structure. Theoretically, with the addition of a patient's cells the matrix will take the form of the patient's own tissue, lowering the risk of rejection. Also with the ability to "print" the collagen matrices on site, there will be a minimal harvest-to-transplant time, greatly reducing the risk of infection. The use of the collagen nanoloom will reduce many of the negative effects of the existing processes of tissue repair.

Tissue Transplants

Most cornea transplants come from human donors. Human donors are scarce because the cornea must be surgically removed from deceased patients no more than 12 hours after death. These corneas must go through testing to see if they are suitable for transplanting, and not all the donor corneas are accepted by the recipients after surgery. After 1 year, about 8.8 percent of the corneas fail. After 4 years, about 20 percent fail [6]. Some people, like Pat Canelli of Lawrenceburg, Ohio, have repeatedly rejected human cornea transplants. "My body rejected human tissue transplants four times [5]." Human transplant methods don't seem to work for these people.

Artificial corneas have been created by Argus Biomedical in Australia. Their cornea, named AlphaCor, is 7 mm in diameter and is made of polymer plastics. This may potentially be a suitable replacement for people like Pat Canelli, but issues with this cornea still exist. "The problem in the past has been that the area where the plastic meets the tissue can break down, which can lead to infection and loss of the eye [5]." After time, these corneas may get scratched up like contact lenses, rendering the corneas useless. An artificial cornea that is more biologically compatible would have a higher chance for the body to accept.

Collagen Production Problems

Currently, collagen production advances have only grown to a level in which artificial skin grafts can be readily made. Skin is a relatively simple structure compared to corneas and the organization of the skin matrices is not as important because the skin does not have to support much tensile load nor does it need to be clear. The majority of collagen structures, such as ligaments, tendons and the cornea of the eye, are required to carry higher loads. Highly-organized collagen matrices can not be developed using current technology.

Artificial skin grafts are advantageous to autografts and allografts because of the higher availability. Severe burn victims seldom have skin to transplant from themselves and allografts have higher rejection rates mostly due to infection [1].

Artificial skin grafts are grown using fibroblasts which are added to cold collagen. The compound is allowed to rise to body temperature and the collagen will gel. As it gels, the fibroblasts arrange themselves around the collagen, generating the growth of new skin cells [1]. There is no control over this self-assembly method because the collagen randomly assembles within the gel (See Figure 3).



Figure 3: SEM of a Collagen Matrix lying on a Silicon Wafer [11]

The cornea of the eye consists of three layers, the epithelium, stroma, and endothelium. The epithelium, or outer part of the cornea, protects the eye from trauma and bacteria. The epithelium layer is five to seven layers thick and is regenerative. The endothelium is a single celled layer at the back of the cornea. The stroma is the thickest layer of the cornea and consists of around five hundred thin layers of collagen fibrils [2]. Current medical advances have allowed scientists to create the epithelium and endothelium layers. The epithelium can be created in culture. The endothelium layer, which doesn't replicate naturally, has been stimulated to also grow in cultures. The stroma layer is the hardest part of the

cornea to create, "The stroma, too, has yet to be replicated in any laboratory [2]." Most scientists who have tried to re-create a stroma by putting stromal cells into collagengel have found that they do not have the strength and clarity of natural ones [2].

The structure of the corneal tissue has a direct impact on the clarity. "Transparency of the corneal stroma depends particularly on the degree of spatial order of its collagen fibrils, which are narrow in diameter and closely packed in a regular array [3]." A certain pattern must be kept in order to correctly refract light into the pupil of the eye and because cells only have a limited ability to rearrange collagen fibrils, they must be as similar as possible to the original structure when formed.

Dr. Jeffrey Ruberti has developed a way to build a stroma by dripping pure collagen onto a warm disk spinning at high RPMs. "This causes collagen to lie down and self-assemble into oriented fibrils that mimic the normal healthy stroma [2]." This method has only produced two fibril layers out of five hundred in a normal stroma. Though it's a step in the right direction, it still isn't sufficient enough to create artificial corneal stromas. New systems must be developed in order to advance in this study.

DESIGN OVERVIEW

Collagen Nanoloom Theory

The goal of the Collagen Nanoloom design is to provide a reliable means to recreate the conditions found in cell surface crypts, where collagen is synthesized. This will be accomplished through thermal control, motion control and pH control. The theory behind this design is based on the existing cell surface crypts (See Figure 4). The Bovine collagen monomers will be stored in a cold reservoir and in a low pH solution to prevent the monomers from self-assembling. The monomers will then travel down a 60 nm diameter channel into a gel maintained at a higher temperature and pH (See Figure 4). [8] Here they will assemble in a highly aligned matrix and adhere to a substrate mounted on the nano-controlled stage.





Design Specifications

Design Specifications						
Component	Specifications	Comments				
1) Printer Head						
a) Collagen Reservoir	Temperature 4°C±1°C pH Maintained at 3.5	pH will be controlled outside of system				
b) Collagen Monomer Concentration	3.0 mg/ml	Concentration will be controlled outside of system				
2) Membrane						
a) Membrane Size	2mm by 2mm					
b) Channel Length	60 nm					
c) Channel Diameter	50-100nm					
3) Gantry/Stage						
a) Assembly Surface	Temperature 37°C±1°C pH Maintained at 7.4	pH will be controlled outside of system				
b) Substrate Velocity	0.01-0.3 micron/sec	Equal to the rate of Fibrils transported down the channels				
c) Motion Step Size/Resolution	40 nm					
d) Minimum Stage Size	25.4mm by 25.4mm					
e) Vibration Isolation	<100nm	Keep to a minimum to prevent damage to tissue				

The design requirements for the Nanoloom are outlined on Table 1.

Table 1: Design Specifications

Design Breakdown

The design is broken down into three sub-systems: the cold zone temperature and chip membrane which consists of the polycarbonate membrane and the membrane housing, the hot zone temperature control, and the motion control which includes the printer head and gantry.

Cooling System / Printer Head – 1st Design

There were three cooling devices that were taken into consideration for the cooling system configuration, which needs to maintain the environment at 4° Celsius. A cold water bath, a water jacket, and a thermoelectric heat pump (Peltier cooler) and all have their advantages and disadvantages in cooling. A cold water bath would need to be attached to an external cooling device such as refrigeration, and although it would maintain the most uniformity in temperature, it would be impractical. A water jacket would need refrigeration outside of the cooling block to obtain the desired temperature before it is to be pumped through the block. This causes added vibrations with the additional flow but is more space saving than the water bath. The water jacket also has cooling limitations because it is a passive cooler. As the

water flows in, it will absorb energy without being able to dispel it until it exits the block so the temperature of the collagen will increase the further along it flows. A Peltier cooler, or thermoelectric cooler, is a heat pump shaped as a rectangular prism. When it is excited by electricity, it starts to pump heat from one side to the other creating a "cold" and "hot" side. This has an advantage over other types of cooling such as refrigeration because of its small size and lack of moving parts and is the reason why it was chosen to cool each of the designs.

The first design has the Peltier cooler is mounted with the cold side down, which will maintain the temperature of the collagen solution and remove the heat being emitted by the substrate. The collagen solution will flow between the cold side of the Peltier and the insulation material. The insulation is meant to provide a barrier between the cold collagen solution and the warm substrate (See Figure 5). The flow rate of the collagen solution through the chip will need to be fast enough to remove some of the heat being emitted through the membrane.



Figure 5: Printer Head Design One

Cooling System / Printer Head – 2nd Design

After further evaluating of the first chip membrane design, it was concluded that by having a device strictly on top of the plate, a temperature gradient would be eventually produced across the entire plate. Thus, the temperature of the collagen flowing would be too inconsistent and collagen fibrils traveling alongside the membrane may start to self assemble.

In the second configuration, the Peltier cooler is mounted with the cold side up to maintain the temperature of the collagen solution (See Figure 6). This allows the Peltier to dump the heat from the hot side to the preheated substrate. The collagen solution will flow over the cold side of the Peltier, down into a reservoir and back out. The insulation will isolate the membrane from the heat generated by the hot side of the Peltier. A thermally conductive material will be used to remove the heat generated by the Peltier to the surroundings and substrate. Unlike the first option for the chip membrane, the flow rate of the collagen

solution through the chip will be slower and hence allow more time for the monomers to permeate through the membrane.



Figure 6: Printer Head Design Two

Cooling System / Printer Head – 3rd Design

There were short comings of the second design. The performance of the Peltier may also be affected because of the stagnant heat that collects underneath the hot side of the cooler. Also, by having the cooling device below the collagen plate, the Peltier would dump heat to the warm environment which would disrupt the temperature of the warm environment. It would not be favorable to dump exorbitant amounts of heat into the warm, thermally-controlled environment. With this design, a temperature gradient would eventually arise like the first design.

Because the previous two designs would not allow for discrete temperature control, a third design was created. In the third design, two Peltier coolers would be used. The TETECH TE-195-1.0-0.8 Peltier cooler was the adopted cooling device and its dimensions are 50x25x3.1 mm with an energy transfer rating of up to 86 Watts. The Peltier coolers will be oriented such that top to bottom will be 50mm and side to side will be 25mm. Installing the Peltier coolers vertically will allow heat to be dumped along the sides of the system.

This is favorable to the top-of-plate Peltier design because there is less area with a temperature gradient. There is also less total area that is exposed to the warm environment, therefore keeping the collagen block as cold as possible. This design also allows for the use of insulation to separate the hot and cold environments.

In order to keep the surfaces touching the collagen as uniform as possible, copper plates will be used to contain the collagen. Two copper plates will "sandwich" a precisely cut gasket to form a channel that will circumnavigate a center gasket. Copper was chosen because of its high thermal conductivity, 391 W/m*K, but because the collagen solution consist of hydrochloric acid and most metals corrode due to the acid, the copper plates will either have to be gold plated or Teflon coated to resist corrosion. The gasket

material specified was Viton, which was selected because of its high tolerance and compatibility to hydrochloric acid.

The gasket part of the third design will actually be three separate gaskets aligned to the copper plates with alignment pins. It must be precisely cut with very strict tolerances in order to achieve the flow desired across the membrane. Other designs considered for the channels were a two piece block of copper or stainless steel that would have a milled channel in each block, put together to form a tube. This method was abandoned due to the difficulty in machining and also problems in sealing.

The insulation layers will be made of ABS molded plastic. This particular material was chosen due to its low thermal conductivity value, 0.128-0.19 W/m*K, high availability and compatibility with hydrochloric acid.

Final designs for holding the assembly together have not yet been realized in the third design, but designs being considered include clamping the assembly together, having numerous rings around the system, and creating a sleeve for the assembly to fit into. The track-etched membrane will simply be glued to the insulation. The insulation could also potentially be glued to the copper block and gasket. Further considerations must be taken before selecting a final design, but the third design can be seen in Figure 7.



Track-Etched Membrane

Figure 7: Cross Sectional View of Printer Head Design 3

Cooling System / Printer Head – 4th Design

The third generation design has been modified and improved upon. The previous design didn't account for fastening, as that was an ongoing process, but the forth generation is near finalization and takes almost all of the components into account.

In addition to the third generation design are two heat sinks that will allow for 3.6 Watts of heat loss each due to natural convection (using an approximated heat transfer value of 8 W/m*K). The "L" shaped aluminum mounting plate (the highest bar in Figure 9) was also added to the cooling assembly to connect the Z-motion stage which will be discussed in the motion control section. The mounting plate will also clamp onto another aluminum plate to hold the assembly together. A peristaltic pump will force the collagen solution to enter either of the tube inlets and the other tube will carry recycled collagen into a reservoir.

There were modifications done on the gasket design to decrease the difficulty of cutting such a small part. The insulation surrounding the gasket and copper plates were extended to provide more performance. The copper blocks were modified as well so the maximum amount of compression on the gasket can be limited. Instead of having two similar copper plates, one plate will have a 2 mm deep cutout spanning the size of the gasket and the other will be flat. The finalized design can be viewed in Figure 8.



Figure 8: 4th Generation Cooling System

Collagen Collector Heating System Design – 1st Design

The warm side of the thermal control system will need to be maintained at 37°C. This will be accomplished by using a combination of a water bath and a heater to ensure an even distribution of heat across the substrate. The heater will keep the water bath at the desired temperature. A secondary bath will be placed into the main water bath. This secondary bath will contain the substrate and a polyvinyl buffer solution, PBS. The warm water and the PBS solution will not be mixed because of the separate dual stage bath (See Figure 9).



Figure 9: Water Bath Schematic

Collagen Collector Heating System Design – 2nd Design

The original design of the collagen collector involved a large water bath that would have been heated to 37°C. This, like other water baths, would have created uniformity in temperature but size and weight constraints have made this design infeasible.

Circular-tube, Watlow cartridge heaters were specified for the second design instead. A relatively large copper block (to distribute heat uniformly) will be drilled with 6.5mm holes to allow for the insertion of the cartridge heaters. Each cartridge heater has an output of up to 120 Watts. Surrounding the copper block will be ABS molded plastic to insulate the sides and bottom of the heating block in order to prevent heat loss (See Figure 10).



Figure 10: Cross Sectional View of Collagen Collector

Collagen Collector Heating System Design - 3rd Design

The second design was big and response times to adjusting temperatures would be mediocre. Having such a large copper block would create uniformity at a cost of lag times so other heating options were explored.

The third design for the heating system was designed around the Minco HK5482R172 heating foil. The foil is 1 mm thick and is more uniform than the cartridge heaters since it is spanned out, 82.5 x 82.5 mm. The height of the insulation and the copper block were reduced to increase the responsiveness of the entire heating system. Height reduction could be implemented now because of the higher uniformity of the heating foils. The top of the copper plate will be insulated by ABS with a cutout to mount the 91 mm square Petri dishes which been purchased. Figure 11 shows a cross sectional view of the collagen collector.



Figure 11: Minco Kapton Heater Design

Thermal Control / Temperature Feedback

Omega temperature controllers will be used to monitor temperature along the top of the tracketched membrane of the printer head and on the surface of the Petri dish on the heating block with feedback coming from J-type thermocouples. The Omega CN77342 PID controllers will cycle the Peltier coolers and the thermofoil heater on and off in an effort to create a stable 4°C on the surface of the track-etched membrane and 37°C in the PBS solution, respectively.

A 24 volt power supply was purchased for powering the Peltier coolers and a variable transformer with a rating of 0-140 volts AC will power the thermofoil heater. Because of the flexibility of the thermofoil heater, the voltage can be adjusted to achieve up to 114 watts from 140 volts or 1 watt with 13 volts inputted. This will be useful to keep the PBS solution at steady-state conditions.

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Omega temperature controllers will be used to monitor temperature along the top of the tracketched membrane of the printer head and on the surface of the Petri dish on the heating block with feedback coming from J-type thermocouples. The Omega CN77342 PID controllers will cycle the Peltier coolers and the thermofoil heater on and off in an effort to create a stable 4°C on the surface of the track-etched membrane and 37°C in the PBS solution, respectively.

A 24 volt power supply was purchased for powering the Peltier coolers and a variable transformer with a rating of 0-140 volts AC will power the thermofoil heater. Because of the flexibility of the thermofoil heater, the voltage can be adjusted to achieve up to 114 watts from 140 volts or 1 watt with 13 volts inputted. This will be useful to keep the PBS solution at steady-state conditions.

Thermal Analysis between the Printer Head and Collagen Collector

Purpose

Performing the thermal analysis between the printer head and collagen collector is important in determining and verifying specifications of the printer head and collagen collector. One specification is the materials selection and dimensions, such as the thickness of the insulation for the printer head and the thickness of the collection solution. The analysis also confirms the temperature settings of the surface of the copper layer and the Peltiers. The Peltiers maintain the temperature of the collagen solution at the optimal temperature of 4°C. The temperature of the copper layer determines the temperature at the bottom of the membrane, which needs to be held at 37°C. The thermal analysis establishes the best flow rate for the collagen solution to help assist the cooling system in maintaining the temperature of the system. It is important to maintain the temperature of collagen solution within the printer head so that the collagen does not assemble within the printer head.

Performing the thermal analysis of the collagen flowing over the membrane will determine the temperature gradient over the membrane. The temperature gradient will determine the amount of recycled collagen that will assemble along the membrane that does not pass through the membrane at a certain flow rate.

Process

The collection solution and printer head are broken up into volumetric nodes. Nodes 1 through 36 are in the first layer of nodes. It is at the center of the printer head, where the collection solution flows under the membrane, see Figure 13. This layer is in the X-Z direction. Since there are flows in the X-direction, nodes in the X-Z directions are not symmetric. More layers were made in the Y direction, see Figure 14. Since the nodes in the Y-Z direction are symmetric, only half of the nodes are needed for the thermal analysis.





	133-138	99-104	65-70	26-29]
	126-132	92-98	58-64	22-25	Q			
	119-125	85-91	51-57	15-21	DY.	mmeu	пс	
	112-118	78-84	44-50	8-14				-
	105-111	71-77	37-43	1-7				
ſ								
AIR		COLLAG	EN	MEMBRAN	E	INSULATION	J C	OPPE

Figure 13: Node Placement of Thermal Analysis of Collection Solution and Printer Head in the X-Z Direction

Calculating the Peclet Number, will establish whether conduction or convection is the dominant mode of heat transfer for nodes with flow, see Eq.1 in Appendix 1. An energy balance equation (Eq. 2 and 3 in Appendix 1) is derived for each node in each dimension, x, y, and z axis, see Figure 14. A finite element circuit analysis can be completed using the energy balance for each node. The circuit analysis uses conductance of each node to connect each node with adjacent nodes in all 3 dimensions. There are two main types of conductance between nodes. One consists of both nodes composing of the same material, see Eq. 4in Appendix 1. The second is when nodes consist of two or more different types of materials. When this occurs, a circuit analysis between materials is needed. See Eq. 5 in Appendix 1. The conductance can be entered into the energy balance equation once each conductance has been calculated in all three dimensions of the node.



Figure 14: Nodal Analysis of Typical Collection Solution Node

The Nodes that represent the collagen solution or collection solution contain a thermal contribution for the flow in the energy balance, see Eq.2 in Appendix 1. Since there is no collection solution flowing through the layer at which the membrane is located, the flow term can be neglected for that layer. A linear temperature profile between fluid nodes will determine the incoming and outgoing temperatures of the flow in the direction of the flow (Eq. 6 and 7 in Appendix 1).

The energy balance equation for each node was inputted into Excel to form two matrices. One matrix equals the unknown conductivities and the other equals the known conductivities. To determine the temperature at each node, the known conductivities must be multiplied by the inverse of unknown conductivities. Since excel can not invert matrices that are larger then 52 rows and 52columns, the matrices were exported to MatLab for this final calculation. This gives the temperatures of each node. See Appendix 1 for all equations.

Results

The Peclet Number for the collection solution came out to be 0.0248, which is much less than 1. Therefore, convection is not a dominant mode of heat transfer for the collection solution. For the collagen solution the Peclet Number equals 282.85 and much larger then 1. Therefore convection is the dominant medium for heat flow. The preliminary thermal analysis was done in excel (see Appendix 2). A temperature profile was done between node 13 and 8, which are the nodes from the copper block to the membrane, see A.2.8. Apendix. A temperature of 39°C will be needed at the copper block in order to

achieve a temperature of 37°C at the bottom of the membrane. However the analysis is not yet complete. Larger numbers of smaller nodes were created to assist in producing more accurate temperatures, 138 nodes. However troubles with the collagen solution nodes prevented a completed finite element analysis within the time frame.

Motion Control System – Phase 1

A nano-motion stage will be use to control the velocity and position of the substrate relative to the printer head, which will be positioned over the substrate. All three systems will be enclosed within a simple scaffolding to minimize any undesired relative motion between the printer head and the substrate. The assembled system will be placed on an air table to minimize vibration. (See Figure 15)



Figure 15: Schematic of Collagen Nanoloom

Motion Control System - Phase 2

Along with resolution, the most important parameter for the X-Y motion system is the velocity stability which determines the smoothness of motion at a low velocity. This is important because although the required velocity of the substrate will be determined during testing, it will most likely be in the range of 10 nm/s to 300 nm/s. Because of this, the worst case will be used to determine the parameter (@10 nm/s ± 5 nm/s). This will ensure that the velocity will be relatively stable within the range. In order to achieve the desired velocity stability and resolution within 25mm of travel an X-Y motion system with a direct linear motor drive would be the best choice. Unlike many stages that utilize a lead screw, a direct linear motor drive would eliminate backlash and windup.

To select the optimal system for X-Y motion control a simple trade study was performed (See Table 2). All relevant parameters; velocity stability, resolution, price per axis, and travel are arranged in

order of importance and given a value of importance 40, 30, 20, and 10 respectively. The values for each parameter are than plotted linearly against a scale of 0.0 to 1.0 and a value is obtained for each individual parameter. The products of the values and the respective importance values are then summed up and a total score is produced. The stage with the total value closest to 100 is the best overall choice.

Requirements >>>>		@10nm/s ±5		5nm	5nm 6500			25mm		
Company	Model #	Velocity Stability	40.0	Resolution	30.0	\$/axis	20.0	Travel (X/Y)	10.0	Total Score
Newmark	NB4-2.5	@10nm/s ±?	0.50	30nm	0.29	1215	1.00	63mm	1.00	
			20.00		8.55		20.00		10.00	58.6
Aerotech	ALS130	@10nm/s ±6	0.80	5nm	1.00	5000	1.00	25mm	1.00	
			32.00		30.00		20.00		10.00	92.0
Aerotech	ABL1000	@10nm/s ±3	1.00	1nm	1.00	8000	0.57	25mm	1.00	
			40.00		30.00		11.42		10.00	
Primatics	PCR34	@10nm/s ±?	0.60	10nm	0.86	7000	0.86	25mm	1.00	
			24.00		25.71		17.14		10.00	76.9
				Importance		Value		Score		

Table 2: Trade Study of X-Y Direction Nano-Motion Devices

The requirements for the Z positioning system are not as strict as for the X-Y motion system. The Z position is a one time adjustment and for this application a lead screw driven positioning stage will be sufficient. The advantage of using lead screw driven positioning stage rather than a linear motor drive is that no counterbalancing is required when using the lead screw positioning stage. To select the best Z positioning system a trade study not unlike for the X-Y system was used (See Table 3).

Requirements >>>>		50nm		10mm		
Company	Model #	Resolution	30.0	Travel (X/Y)	20.0	Total Score
Newmark Systems inc	NB4-2.5	30nm	1.00	63mm	1.00	
			30.00		20.00	50.0
Aerotech	ATS50	50nm	1.00	25mm	1.00	
			30.00		20.00	50.0
Primatics	SRB50	100nm	0.10	60mm	1.00	
			3.00		20.00	23.0
		Importance		Value		Score

 Table 3: Trade Study of Z-Direction Nano-Motion Devices

Gantry Design

The Nanoloom Gantry has few requirements that must be met to optimize the performance of the machine as well as increase the ease of use. The first and most important criteria are the rigidity and stiffness requirement. The Collagen Nanoloom will be used in a location where it will be subjected to time variable ambient vibrations. There is a great need to minimize the relative motion of the printer head to the

substrate to maximize the printing accuracy of the Nanoloom. The targeted upper limit for vibration amplitude and relative motion between printer head and substrate is one hundred nanometers or lower. Although the Nanoloom will be mounted on a vibration damping air table, a worst case approach must be taken when designing the gantry.

Materials were considered as a preliminary phase of the design process. The ideal material for the gantry would have a high Modulus of Elasticity to assure that when under the highest of stresses, the deformation of the gantry will be minimized. As seen in Hooke's Law, a high modulus of elasticity will minimize the deformation within the system. With a modulus of elasticity of approximately 205 GPa, and a relatively low cost, steel is an attractive option for the gantry material.

Secondly, relating to the rigidity requirement is the method for fixing the printer head. It would be beneficial to minimize the length of the arm connecting the printer head to the z-stage. This would minimize the relative motion of the printer head to the x-y stage. In a preliminary design the gantry was a half box, seen in Figure 17 below. The concern with this design is that in mounting the z-stage on the rear plate of the box, the support arm connecting the printer head to the stage would need to be lengthy to center the printer head over the zero position of the x-y stage. This was approached by translating the z-mount plate forward, positioning the printer head over the x-y stage with minimal arm length as seen in Figure 18 below.



Figure 16: Gantry Design I

In addition to eliminating vibrations within the system, there are optional requirements that are not essential to the functionality of the Nanoloom, but important for the ease of use of the machine. The first optional requirement is to have an open, easily accessible front stage mounting area. This will include having clearance on all sides of the x-y stages for any adjustments that need to be made to the stages. It would also be beneficial to have the system be modular to make maintenance quick and simple. The printer head and collagen collector must be accessible for any repairs required (See Figure 17 and 18). These requirements will be considered in the design, but the rigidity requirement holds the most weight in the design decision making process.



Figure 17: Gantry Design II

The final design incorporated the aforementioned half box with translated z-stage mount. This, again, can be seen in Figure 18 above. To maximize the rigidity of the system a high modulus material had to be chosen. After exploring many possibilities, a decision of 304 Stainless Steel was made with mating done welding. With its Modulus of 200 GPa., 304 it would assure a rigid structure at relatively low cost. An ANSYS simulation was an essential tool in validating the design of the gantry. The first analysis that had to be completed was a modal analysis of the gantry to determine at what frequency the structure would resonate at. The gantry will be mounted on a Newport Air Vibration Damping table that will

significantly damp vibrations exceeding a frequency of 10 Hz. The modal analysis revealed that the first resonant frequency was at 321.76 Hz which is well above the table lower limit of 10 Hz. This resonant frequency, along with the second through fifth resonant frequency can be found in Table 4 located below.

Gantry Resonance Frequencies					
1	321.76 Hz				
2	846.12 Hz				
3	929.88 Hz				
4	1184.5 Hz				
5	1256.3 Hz				

Table 4: Gantry Resonant Frequencies

The next analysis that was required was to find the relative motion of the printer head mount (zstage mount) to the base of the gantry (or the x-y-stage mount) to assure that the amplitude of vibrations did not exceed a magnitude of 100 nm. This is an essential design criterion because during Nanoloom operation, if the system encounters low frequency vibrations, any large relative motion of the printer head to the stage may stress the collagen fibers being printed and damage or tear the specimen. A harmonic analysis was run in ANSYS to observe relative motion of the printer head mount to the base of the gantry. In this analysis the gantry was oscillated in x, y, and z directions at a max amplitude of 1mm, which is a worst case assumption, at frequencies between 0 and 60 Hz, which are common for ambient vibrations. The displacements at a node at the center of the base of the gantry were compared to the displacement of 35 nm occurred in the system at a frequency of 60 Hz. This maximum displacement occurred in the x-plane of the stage, but well exceeds the design requirement of 100 nm. The plots of the x, y, and z displacements are plotted below in Figures 19-21.



Figure 18: X-Directional Gantry Deflection @ Printer Head Mount



Figure 19: Y-Directional Gantry Deflection @ Printer Head Mount



Figure 20: Z-Directional Gantry Deflection @ Printer Head Mount

The results of the ANSYS simulation were all acceptable in validating the design of the gantry. The Structure will be capable of isolating the low frequency vibrations that it may encounter and minimize the relative motion of the printer head to the stage assuring high printing quality.

RESULTS AND FUTURE RECOMMENDATIONS

The Nanoloom was assembled and tested on December 7, 2005. Not all the requirements were met, and collagen was not tested, but recommendations are made on how to fix the problems.

Accomplishments

The heating system was fairly well controlled. Temperatures could stay within ± 0.75 °C of the input amount but even tighter tolerances could possibly be achieved with fine tuning of the PID controllers. Gantry analysis in ANSYS proved that the gantry would not resonate until 321.76 Hz, exceeding the 10 Hz requirement and amplitudes would stay below 100 nm for reasonable vibrations within that range. Motion control was kept within the set range with the purchase of the Aerotech motion control stages.

Problems Encountered

The Nanoloom didn't meet the cooling requirements. The Peltier-cooled printer head could not maintain a temperature under 10°C, well off of the 4°C requirement. During testing without collagen, we had achieved temperatures as low as 3.5°C at the copper but with the thermocouple collecting data from the printer head, temperatures of 8.0°C were the best results we could achieve, and only for a moment. The problem with the design is that there is inadequate cooling of the heat sinks, restricting the Peltier from pumping out more energy. Peltier coolers can only achieve a certain difference-in-temperature and because

there is not enough heat convection over the heat sink, the temperature inside the printer head will slowly creep up. Without flow, a temperature of 12.0°C can be maintained inside the printer head. Results of tests can be seen in Table 4.

Head Stagnant in Water						
Flow Rate		T_{cold}	T _{hot}			
175	cc/hr	17.6	38.5			
82	cc/hr	19	37			
1.75	cc/hr	19.9	37.2			

Head Moving in Water (.1 mm/s)					
Flow Rate		T _{cold}	T _{hot}		
175	cc/hr	17.6	38.5		
82	cc/hr	19	37		
1.75	cc/hr	19.9	37.2		

Table 5: Temperature of Printer Head submerged in water

Future Recommendations

The cooling problems are difficult to solve. The biggest issue was that there was not enough airflow flowing over the surface of the heat sinks. With better cooling, possibly with a water jacket, temperatures of 4°C can be achieved. A DC transformer or entire PID system for the Peltier coolers should also be purchased. There is no control over the cooling rate with the dedicated power supply. Using the Omega PID controllers may also have a detrimental effect on the life of the Peltier coolers. Since there is going to be a computer to control the motion system, using the PID controller from TETECH may not be a bad option, except that the thermocouple would need to be replaced by a thermistor. Other companies should have thermocouple PID controllers however. In addition, better materials for insulation could also be used to keep the temperature more stable.

Conclusions

Though the device didn't fulfill all the requirements, many of them were still achieved. Getting a temperature gradient of 33°C through a membrane that is only 100 micrometers is a difficult task to achieve, and getting a 17°C gradient was an accomplishment. Being able to integrate all the components on a constrained time-frame was also an achievement. A strong framework has been laid out and the group feels that with just one more week, we could have achieved all of our design goals. Through all of the late nights and early mornings, the hours spent in front of the computer and at weekend meetings, overall, we can look back and feel proud of our accomplishments knowing that we had one of the more difficult Capstone design projects this year.

A.1.1Peclet Number, Pe

Variables

- Heat Transfer Rate by Conduction q_{cd}:
- Heat Transfer Rate by Convection q_{cv}:
- Flow Velocity of Fluid V:
- Density of Fluid ρ:
- Specific Heat of Fluid c:
- L: Channel Length
- k: Conductivity of Fluid

Equation

$$Pe = [q_{cd}/q_{cv}] = [V\rho cL_{ch}/k]$$

A.1.2 Nodal Energy Balance Equation

Variables

- q: T Heat Transfer Rate
- Temperature of Node
- C: Conductance Value
- V: Flow Velocity of Fluid
- Density of Fluid ρ:
- Specific Heat of Fluid c:
- A: Area

Equations

 $E_{in} = E_{out}$

 $q_{in} = q_{out}$

$$q_{x,i} + q_{y,i} + q_{z,i} + \rho cVAT_{in} = q_{x,o} + q_{y,o} + q_{z,o} + \rho cVAT_{out}$$
(Eq.2)

$$0 = q_{x,i} - q_{x,o} + q_{y,i} - q_{y,o} + q_{z,i} - q_{z,o} + \rho cVA[T_{in} - T_{out}]$$
(Flow Contribution)

$$0 = Cx_{,o}(Tn - Ti) - Cx_{,i}(Tj - Ti) + Cy_{,o}(Tn - Ti) - Cy_{,i}(Tj - Ti) + Cz_{,o}(Tn - Ti) - Cz_{,i}(Tj - Ti) + \rho cVA[T_{in} - T_{out}]$$

$$0 = \sum C_{i,j}(T_j - T_i) + \rho cVA[T_n + T_i/2 - T_i - T_j]$$

$$0 = \sum C_{i,j}(Tj - Ti) + \rho cVA[(Tin) - (Tout)]$$
(Eq.3)

(Eq. 1)

A.1.3 Conductance Equations

- Variables
- k: Conductivity A: Area
- Δx : Displacement

$$\frac{\text{Equations}}{C = (k * A)/\Delta x}$$
(Eq.4)

$$C = [(((k1 * A)/\Delta x1)^{-1}) + (((k2 * A)/\Delta x2)^{-1})]^{-1}$$
(Eq.5)

A.1.4 Temperature Profile



Meterial	Thermal Conductivity (W/m*K) Constant		Specific (J/kg*	Heat K)	Densi (kg/m	ty ³)
Material	valu	e	Constant	value	Constant	value
Cu	K _{cu}	385	Cp _{cu}	385	ρ _{cu}	8960
ABS	K _{abs}	0.19	Cp _{abs}	2000	ρ_{abs}	1050
Viton	K _{viton}	0.14	Cp _{viton}	450	ρ_{viton}	1900
Polycarbonate						
(membrane)	K _{mem}	0.72	Cp _{mem}	1820	ρ _{mem}	1020
Water						
(Solution)	K _{h20}	0.6062	Cp _{h20}	4182	ρ _{h20}	998.23

A.2.1 Thermal Properties of Materials

A.2.2 Displacement length of the nodes in x, y and z direction

Length (m)	Length in x	Length in y	Length in z
Α	0.001	0.002	0.003
В	0.0005	0.00435	0.001
С	0.00435	0.003	0.0001
D	0.00715	0.036	0.0005
E			0.0025
F			0.0015

A.2.3 Calculating Conduction Constants for node 6 to 10

	Nodes	6	7	8	9	10
ıt	Δx1 (m)	0.000575	0.000575	0.000225	0.000225	0.000575
	Δx2 (m)	0.000125	0.000125		0.0001	0.000125
Jer	Δx3 (m)		0.000225		0.000575	
en	Δy1 (m)	0.000225	0.000225	0.000225	0.000225	0.000225
lac	Δy2 (m)					
sp	Δy3 (m)					
ā	Δz1 (m)	0.00055	0.00055	0.00055	0.0005	0.00055
	Δz2 (m)	0.00005	0.00005	0.00005	0.00005	0.00005
	Δz3 (m)	0.000125	0.000125		0.000125	0.000125
a	Area in x (m ²)	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002
٨re	Area in y (m ²)	1.15E-07	0.00000025	0.0000002	0.00000025	1.15E-07
ব	Area in z (m ²)	0.0000023	0.00000005	0.00000004	0.00000005	0.0000023
_	Cxin	2.1085E-05	1.5435E-05	0.000064	0.000064	1.7823E-05
nductior	Cxout	1.7665E-05	0.000064	0.000064	1.83921E-05	2.1085E-05
	Cyin	0.00030984	0.00008	0.000064	0.00008	0.00030984
	Cyout	0.00030984	0.00008	0.000064	0.00008	0.00030984
ទ្រីខ័	Czin	0.0002535	5.59125E-05	4.09527E-05	5.59125E-05	0.0002535
Ŭ	Czout	0.00031065	6.87437E-05	0.000576	6.87437E-05	0.00031065

	Nodes	11	12	13	14	15
	Δx1 (m)	0.000575	0.0007	0.000225	0.0007	0.000575
	Δx2 (m)	0.0007	0.000225	0.000225	0.000225	0.0007
ent	Δx3 (m)					
em	Δy1 (m)	0.000225	0.000225	0.000225	0.000225	0.000225
olac	Δy2 (m)					
lisp	Δy3 (m)					
	Δz1 (m)	0.002	0.002	0.002	0.002	0.002
	Δz2 (m)	0.00055	0.0005	0.0005	0.0005	0.00055
	Δz3 (m)		0.00005	0.00005	0.00005	
Area	Area in x (m ²)	0.0000002	0.0000002	0.0000002	0.0000002	0.0000002
	Area in y (m ²)	0.00000115	0.00000115	0.0000002	0.00000115	0.00000115
	Area in z (m ²)	0.00000023	0.0000023	0.00000004	0.0000023	0.0000023
	Cxin	0.000210852	5.42857E-05	0.00064	5.42857E-05	0.000210852
ion Its	Cxout	0.0001732	0.000168889	0.00064	0.000168889	0.0001732
ucti itan	Cyin	0.003098356	0.000971111	0.00064	0.000971111	0.003098356
puq	Cyout	0.003098356	0.000971111	0.00064	0.000971111	0.003098356
ပိပိ	Czin	0.000069713	0.00002185	0.0000144	0.00002185	0.000069713
	Czout	0.000069713	0.000257197	1.48092E-05	0.000257197	0.000069713
on Its	Velocity	3.00E-07	3.00E-07	3.00E-07	3.00E-07	3.00E-07
ecti tan	ρϲVΑ	1.25E+00	1.25E+00	1.25E+00	1.25E+00	1.25E+00
ons	x profile in	0.319444444	1.25	-1	-0.069444444	
ပိပိ	x profile out		0.319444444	1.25	-1	-0.069444444

A.2.4 Calculating Conduction and Convection Constants for node 11 to 16

A.2.5 Calculating Conduction and Convection Constants for node 16 to 20

	Nodes	16	17	18	19	20
	Δx1 (m)	0.000575	0.0007	0.000225	0.0007	0.000575
	Δx2 (m)	0.0007	0.000225	0.000225	0.000225	0.0007
ent	Δx3 (m)					
e	Δy1 (m)	0.000225	0.000225	0.000225	0.000225	0.000225
lac	Δy2 (m)					
isp	Δy3 (m)					
	Δz1 (m)	0.0002	0.0002	0.0002	0.0002	0.0002
	Δz2 (m)	0.000055	0.00005	0.00005	0.00005	0.000055
	Δz3 (m)		0.000005	0.000005	0.000005	
	Area in x (m ²)	0.000006	0.000006	0.000006	0.000006	0.000006
Area	Area in y (m ²)	0.000000115	0.000000115	0.0000002	0.000000115	0.000000115
	Area in z (m ²)	0.0000023	0.0000023	0.0000004	0.0000023	0.0000023
	Cxin	0.000632557	0.0005196	0.001616533	0.0005196	0.000632557
on ts	Cxout	0.0005196	0.001616533	0.001616533	0.001616533	0.0005196
tan	Cyin	0.000309836	0.000309836	5.38844E-05	0.000309836	0.000309836
nbn	Cyout	0.000309836	0.000309836	5.38844E-05	0.000309836	0.000309836
ပိပိ	Czin	0.00069713	0.00069713	0.00012124	0.00069713	0.00069713
	Czout	0.002535018	0.00278852	0.00048496	0.00278852	0.002535018
uo	Velocity	3.00E-07	3.00E-07	3.00E-07	3.00E-07	3.00E-07
ecti	ρςVA	1.25E+00	1.25E+00	1.25E+00	1.25E+00	1.25E+00
N v	x profile in	0.319444444	1.25	-1	-0.069444444	0
ပိ	x profile out	0	0.319444444	1.25	-1	-0.069444444

Т6	T7	Т8	Т9		T10		T11		T12	T13
0.001223	-1.77E-05	0		0		0	-0.0002	54	0	0
-1.54E-05	0.000364	-0.000064		0		0		0	-5.59E-05	0
0	-0.000064	0.000873	-0.0	00064		0		0	0	-4.1E-05
0	0	-0.000064	0.0	00367	-1.84	E-05		0	0	0
0	0	0	-1.7	78E-05	0.00	1223		0	0	0
-6.97E-05	0	0		0		0	1.66E+	00	-4.00E-01	0
0	-0.000257	0		0		0	-1.65E+	00	3.22E+00	-1.57E+00
0	0	-1.48E-05		0		0		0	-2.82E+00	1.57E+00
0	0	0	-0.0	00257		0		0	0	-5.43E-05
0	0	0		0	-6.97	E-05		0	0	0
0	0	0		0		0	-0.0025	35	0	0
0	0	0		0		0		0	-0.002789	0
0	0	0		0		0		0	0	-0.000485
0	0	0		0		0		0	0	0
0	0	0		0		0		0	0	0
.						•				
T14	T15	T16		T17		T18		T1	9	T20
()	0	0		0		0		0	0
()	0	0		0		0		0	0
()	0	0		0		0		0	0
-5.59E-05	5	0	0		0		0		0	0
(-0.0002	54	0		0		0		0	0
()	0 -6.9	7E-05	0			0		0	0
()	0	0	-2.1	-2.19E-05		0		0	0
1.25E+00)	0	0		0		1.44E-05		0	0
-8.45E-02	2 8.68E-	·02	0		0		0		-2.19E-05	0
-1.17E+00) -8.03E-	·02	0		0		0		0	-6.97E-05
()	0 1.66	6E+00	-0.4	00585		0		0	0
()	0 -1.65	5E+00	3.2	2E+00	-1	.567091		0	0
()	0	0	-2.8	2E+00	1	.57E+00		1.250763	0
-0.002789)	0	0		0	-{	5.20E-04		-8.07E-02	0.085354
() -2.54E-	03	0		0		0		-1.17E+00	-8.20E-02

A.2.6 Constant Matrix at nodes from 6 to 20

A.2.7 Temperature Matrix Results

	Calculated
Nodes	Temperature
6	29.89121266
7	27.48669744
8	14.1476901
9	27.65780261
10	29.93578174
11	38.76352763
12	38.02132626
13	37.23846011
14	39.00274783
15	38.96796687
16	38.87831852
17	38.49803096
18	38.09733076
19	39.00042158
20	38.99499345

A.2.8 Temperature Gradient Graph at Middle nodes with Copper set Temperature 39° C



Temprature Gradient Down the Middle

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