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ABSTRACT

THE DEVELOPMENT OF A BIODEGRADABLE SCAFFOLD FOR A TISSUE ENGINEERED HEART VALVE

by
Thomas Adam Alheidt

In the current state of medical technology, there exists the need for a quality medical device to replace a failing heart valve. Presently, mechanical valves as well as donor tissue valves, either from humans or animals are used to replace failing heart valves. These valves although they can operate in the heart satisfactorily are not equal to the body replacing its own valve. Tissue Engineering in simple terms is the field of helping the body replace its own failing organ. In scientific terms, Tissue Engineering is a relatively young field, a majority of the major advancements have come in the last ten years. Currently, work is feverishly being done to develop a tissue engineered heart valve both at MIT and at the Harvard Medical School.

In this thesis, the author will detail a group of tissue engineering scaffolds that were developed and tested which are comprised of biodegradable materials. As the quantity of heart valve cells increases the polymer thickness needs to be decreased, or degraded whereby keeping the overall heart valve thickness within its physiological limitations. Also, in this thesis, the author will detail the initial and then final solvent casting process used to develop the test samples. The first process manufactured a three dimensional test sample whereas the final process

was used to develop two dimensional flat rectangular samples. These samples produced from the final processing method showed promising results as well as a manufacturing process capable of producing repeatable results with varying compositions. Finally, the author will detail the recommended design and development paths both with the material and the sample preparation process.

THE DEVELOPMENT OF A BIODEGRADABLE SCAFFOLD FOR A TISSUE ENGINEERED HEART VALVE

by

Thomas Adam Alheidt

A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
In Partial Fulfillment of the Requirement for the Degree of
Master of Science in Biomedical Engineering

Department of Biomedical Engineering

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Approval Page

THE DEVELOPMENT OF A BIODEGRADABLE SCAFFOLD FOR A TISSUE ENGINEERED HEART VALVE

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This thesis is dedicated to my wife, Sharon, and to my daughter, Natalie Rose for without whose help this thesis could not have been done.

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CHAPTER 1

INTRODUCTION

"U.S. surgeons performed over 93,000 surgical heart valve repair and replacement procedures in 2001 (about 300,000 were performed worldwide), yet this market is still severely underserved." [Medtech]

The heart valves being used in these procedures are both tissue and mechanical. While it is widely accepted that they can prolong and improve a patient's life, they are not the perfect replacement for a damaged valve. Patients who have their heart valves replaced with either a mechanical or tissue valve must endure a lifetime of drug regimens designed to keep their body from degrading its function. "Artificial heart valves have been used clinically for nearly four decades. In that time advances in our understanding of cardiac physiologic features, biomechanics and materials science have consistently led to changes in heart valve prosthetics." [Sapirstein] Where early experimental designs failed in both animals and humans from a lack of biomaterial knowledge, now is no longer an issue. "Blood flow through the valve is non-turbulent, and thus hemolysis and stimulation of the clotting cascade are limited. Biocompatibility is paramount: the blood contacting surfaces are not thrombogenic." [Sapirstein] Doctors and engineers first inserted their design in the tricuspid valve, since the pressure and flow rates are lower at that point than at the mitral valve.

The theoretical model proposed in this literature review is the development of a biodegradable heart valve scaffold. The existing heart valves currently on the

market will be reviewed from recent articles, for both mechanical and tissue designs with references made to both positive and negative design aspects. The proposed heart valve is to be created from a bio-degradable scaffold dimensionally equal to that of a human heart valve. Once the heart valve scaffold has been created, it would then be inserted into a bioreactor. Once inside the bioreactor endothelial growth will begin, coinciding with the degradation of the polymeric scaffold. The articles reviewed in this paper support the decision made on the overall design of the scaffold, with emphasis being the material structure and composition. Listed in the literature review conclusion is a list of potential scaffolds materials.

CHAPTER 2

LITERATURE REVIEW

2.1 Tissue valves

Tissue valves being used today represent the best mechanical substitute to the original valve. A weakness of tissue valves, however, is that they have a short lifespan. "Replacement of damaged heart valves with tissue valves has been a clinical option since Ross and Barratt-Boyes first described aortic valve replacement with a homograft in the early 1960s." [Sapirstein] Today there are four types of tissue valves used in the United States: The tri-leaflet valve, porcine heterografts, allograft, and autografts. Only autograft valves do not need to be treated with glutaraldehyde, a chemical process used to sterilize and strengthen the valve. Autograft valves don't need to be either imuno-supressed or strengthened since they will be re-populated with native cells immediately upon insertion, which does not occur with bovine, porcine, or Allograft tissue valves. Tissue valves are chosen for their excellent flow parameters; however, they have a much shorter life expectancy (7 to 10 years) as compared to mechanical valves. While inside the body, the valve can fail due to complications caused by calcification and or leaflet wear. After the valves (bovine or porcine)

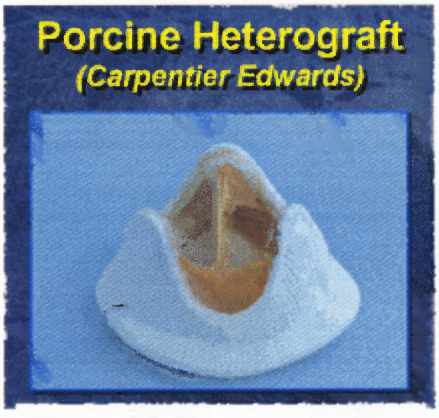

Figure 2.1 Porcine heart valve used in surgery.

have been removed from the animal, they are cleaned and prepped, a ring is attached to the bottom of the valve, and finally it is treated with glutaraldehyde.

As previously stated, tissue valves are treated with glutaraldehyde to reduce the immune response, not eliminate it. Interestingly, in some cases cellular attachment has been reported to both porcine and bovine tissue valves. As with human valves, bovine and porcine heart valves, which are composed of extracellular matrix proteins, can act as intrinsic templates for cellular attachment, even when implanted into humans. "Therefore, various cell extraction techniques are used to create decellularized tissues, including chemical, enzymatic and mechanical methods of removing cellular components, and leaving a material comprised primarily of extracellular matrix components." [Schmidt]

A ring is attached to the valve, which could be composed of either a polyester knit fabric or Dacron. The ring is used to give the surgeon an area to suture the valve to the heart. During the development process, minor modifications are made to the valve leaflets, since their excellent flow characteristics are one of the primary reasons for their usage. At times, the leaflets are supported with stents, to provide added strength. Bovine and porcine valves have been used due to their close physiological composition to the human heart valve.

Tissue valves in general have better hemodynamic qualities than mechanical valves, which results in a lower rate of thrombus formation events. "In order for materials to be transplanted to a patient from a donor, especially an

animal donor, the tissue must be modified to increase resistance to degradation and to decrease immunogenicity, while maintaining natural mechanical properties." [Schmidt] Tissue valves used today are treated with glutaraldehyde, "Glutaraldehyde-treated tissue exhibits altered mechanical properties compared to untreated tissue. Porcine aortic valves crosslinked with glutaraldehyde tend to be stiffer than fresh tissue and have stress relaxation rates about 60% of those for fresh valves. Treated tissues also show increased apparent tensile extensibility associated with shrinkage during fixation". [Schmidt] Glutaraldehyde treatment of tissue valves has the following effects.

1. Suppress the immune response.
2. Increase the strength of the valve through cross-linking, most likely being the collagen already present in the valve.
3. Enhance the materials resistance to enzymatic and or chemical degradation.
4. Sterilize the material.

While this strengthening of the material and increased resistance to physical degradation is advantageous glutaraldehyde treatment also prevevnts the tissue from being repopulated with the patient's cells. "The major drawback to bio-prostheses is the relatively high rate of structural deterioration that almost uniformly occurs. Leaflet wear, exacerbated by calcification, leads to tears and loss of adequate adaptation; clinically important valvular stenosis is less common." [Sapirstein] A drawback from tissue valves is that when they are treated with glutaraldehyde they tend to be more susceptible to degradation and

are also prevented from being remodeled by the body. Cross-linking by glutaraldehyde decreases the display of antigenic determinants by killing viable tissue cells and by controlling the stability of the collagen triple helix. Data collected thus far on tissue valves would suggest that, unless a method can be developed to promote positive cellular growth on the valve after implantation, no dramatic improvements will be made in this technology. A heart valve composed from native tissue, unlike bovine, porcine, and allografts, has the ability to be repopulated with a patient's own cells. This creates a more natural heart valve.

2.2 Tissue engineered valves

Over the past few years, researchers have created tissue scaffolds using biodegradable polymers. These scaffolds are then seeded with endothelial cells. After the cells have been seeded onto the scaffolds, they are placed in a bioreactor for accelerated growth. Once the cell layer has grown to a sufficient thickness, the valve is then implanted into the body. Once the tissue engineered valve is implanted into the heart, it will immediately begin to adsorb proteins and as time progresses, achieve proper endothelial attachment strength and orientation. With acellular tissue, there is no need to treat the tissue chemically, i.e. by glutaraldehyde, to suppress the patient's immune response. There is also no need to physically reinforce the valve with stents so that it remains operational during the numerous cyclic loads during the valve's lifespan. These valves are the latest in heart valve technology. They offer the most promise since they

would enjoy the benefits of both mechanical and tissue heart valves without having any of their detriments.

The development of a tissue engineered heart valve has been hindered in the past due to the lack of quality biomaterials. For a material to be successful as a heart valve scaffold, it must be non-thrombogenic and have elastic and physical properties similar to those of a native heart valve. One issue that needs to be addressed is the following: tissue growth inside the bioreactor must be comparable to natural tissue. Studies have shown that valves exposed to realistic process parameters while inside a bioreactor do have equivalent physical properties.

The dilemma occurs when it becomes apparent that the scaffold material cannot withstand the arterial pressure of a native valve. Currently researchers avoid this issue by using venous blood pressure to test their valves. In the present medical technology field, there are several teams throughout the world trying to develop varieties of heart valve scaffolds. If a heart valve scaffold is going to be successful in the heart valve market, it must be grown under an arterial pressure gradient. Tissue engineered heart valves only capable of operating under venous pressure address a small fraction of the overall need. Testing has been conducted in the field where a single acellular heart valve leaflet was implanted into an existing animal valve while leaving the other two leaflets intact. The other two leaflets thus served as controls in the experiment. This procedure appears promising as a method for testing valve leaflet materials.

2.3 Mechanical valves

The introduction of the first mechanical heart valve occurred in 1952, when Charles Hufnagel implanted his ball in cage mechanical heart valve. Mechanical heart valves are defined by not having any living tissue anywhere in, or on the device. There are currently three designs being marketed which are approved by the FDA. Those designs are the following; ball in cage, tilting disc, and the bi-leaflet design. The valves entered the market in the same order as they were listed.

The first successfully marketed heart valve was the Star-Edwards caged ball heart valve shown in Figure 2.2, a design similar to Hufnagel's and also modeled after a design used by Ellis and Bulbulian. The valve was developed in the 1950s, and clinically used for the first time in August 25, 1960 during a human clinical trial. When Starr and Edwards first began their heart valve development, they were trying to develop a valve that closely mimicked the human heart valve. During this early development work, they attempted a design with two leaflets that were composed of silicone rubber and were hinged to a crossbar made of solid Teflon, which also had a Teflon cloth attached for fixation purposes. "The leaflet valves were plagued by thrombus formation. Thrombus would originate at the suture line and grow by direct extension onto the leaflets. In most cases, the valve became totally occluded after only 2 or 3 days. After

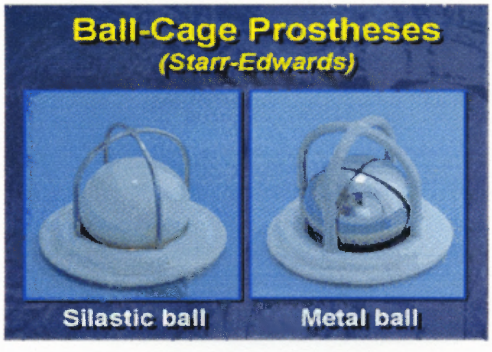

Figure 2.2 Ball-Cage heart valve, first heart valve marketed in the US

months of work, Starr and Edwards abandoned the leaflet valve to go to a ball valve." [Matthews]

The valve developed by Starr and Edwards and used during their initial clinical trial remained essentially unchanged for many years to come. "It had a methyl methacrylate (Lucite) cage with thick struts and a machined ring orifice. A compression molded silicone rubber ball was placed inside the cage and the ring was then solvent welded to the cage with acetone." [Matthews] In medical device development terms it had a very quick development cycle "Lowell Edwards determination and financial backing supported the provision of new models for animal implantation every few weeks or months, allowing the screening of a large number of designs in a short period of time." [Matthews] As a precautionary step barium sulfate was embedded in the poppet, the barium served to make the poppet radiopaque.

Additional design changes were attempted, however, the design implemented during this initial clinical trial and first marketed during the 1960's has been unchanged and used successfully for over twenty years. Although the Starr-Edwards valve is not as heavily used now, as it was in the past, it is estimated that the valve has been inserted into more than 175,000 patients. The positives of mechanical valves are listed below, however the biggest negative of a

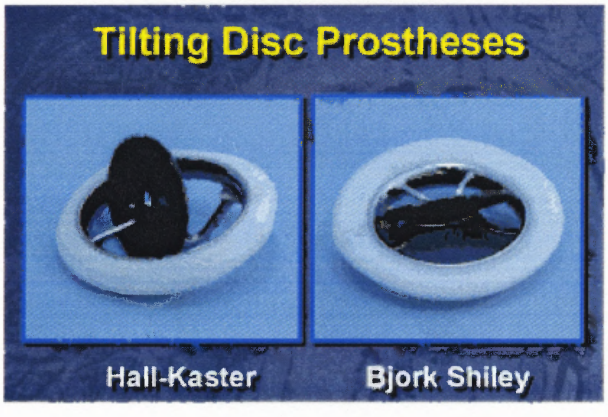

Figure 2.3 Tilting disc heart valve, later recalled from market due to catastrophic failure.

mechanical heart valve is that the valves have a heavy shear rate, and unnatural flow waves. Hence patients with mechanical heart valves need to be medicated throughout their life to guard against thrombus formation. Table 2.1 lists mechanical heart valves that have either been used in the past and or still being used today. Each manufacturer listed in the table is divided into the following three general design classifications: Ball Valves, Bi-Leaflet Disc Valves, and Single Leaflet Valve. The following is a list of benefits that are attributable to mechanical heart valves, but not enjoyed by Tissue Valves.

1. Mechanical valves can be mass-produced whereby controlling their material composition and dimensions.

2. Valves are composed of bio-inert materials, whereby not creating an adverse immune response from the body.

3. Materials used are of high strength, therefore can survive under extreme cyclic loading.

4. Easily implant able

Table 2.1 Mechanical Valves

Ball Valves	Bi-Leaflet Disc Valve	Single Leaflet Valve
Starr Edwards valve	St Jude Valve	The Bjork Shiley valve
Magovern-Cromie Sutureless Valve	Medtronic Parallel Valve	Alliance Medical Technologies Monostrut
Smeloff-Sutter Valve	ATS Bileaflet Valve	The Medtronic-Hall valve
	Carbomedics Valve	Omniscience valve
	Edwards Duromedics Valve	

During the 1970's, a new design was introduced to the market, the "flapper" design, it consisted of a circular disk positioned within a metal ring and then inserted inside a sewing cuff. This single leaflet valve design, refer to figure 2.3, decreased the size of the valve and also reduced the amount of

components, however, as time passed the design was shown to be inadequate. "Mechanistically it functions like a native valve, permitting unimpeded forward flow when open while preventing regurgitant flow when closed." [Sapirstein] The valve did not operate very well while in the body after repeated operation, the strut ended up separating from the rest of the valve, which then resulted in a disk embolization. Following this catastrophic event, the valves were pulled from the market. Attempts were made at improving the design, however, confidence in the design was lost and it never regained its initial momentum. Several other designs were developed that addressed this concern, either by having the struts contiguous with the metal of the valve ring (Technologies Monostrut) or a Titanium shaft that passes through the center of the disk, (Medtronic-Hall) or where the disk operates by hinging against the two sides of the ring, (Omniscience valve). However, the heart valve of choice currently is the bi-leaflet design by St. Jude Medical Inc. This mechanical valve refer to figure 2.4, contains two semicircular disks of pyrolytic carbon that open with independent hinge mechanisms. This design provides the essential design characteristics needed from a mechanical heart valve, long life (almost non-existent structural failures), non-thrombogenic, low blood shear rate, and easy to install during surgery. The pyrolytic carbon valves

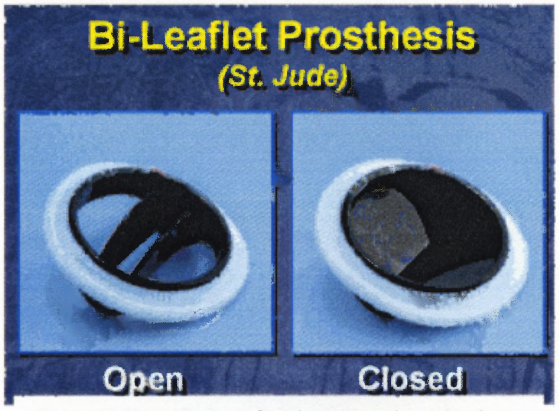

Figure 2.4 Bi-Leaflet heart valve design, marketed by St. Jude, it has the majority market share of the mechanical heart valve field.

are formed through a repetitive heating cycle that gives the material a smooth strong surface resistant to thrombus formation.

Mechanical heart valves unlike tissue valves offer long term durability, however, patients using them need life-long anticoagulation therapy, often suffer from bleeding disorders, and the flow dynamics differ quite significantly from a natural heart valve. Considering all these drawbacks to the mechanical valves, their most important criteria is their durability. Although it can be done, re-operation is not a preferable option, therefore, selecting a tissue valve for a younger patient as the prosthesis of choice is usually denied. Similarly to the tissue valve, mechanical valves are a mature design, and any significant increase in either the tissue or mechanical heart valves effectiveness is highly unlikely. Mechanical valves have roughly twice the amount of years of operational service as compared to tissue valves. "Structural failure of approved valves is almost nonexistent, and valves can reasonably be expected to perform properly for at least 20 years. Anticoagulation with warfarin sodium is mandatory at present. If the level of anticoagulation is maintained at appropriate levels, the rate of valve thrombosis is low (0.1 to 0.3 events/100 patient-years)." [Sapirstein]

During 1968, the engineers that helped to develop the first Starr-Edwards heart valve described the "nine commandments" that they used to help guide them through their development cycle. The nine commandments focus on the following characteristics, which they deemed to be of high concern, these commandments are an excellent basis for a heart valve designer to follow.

1. Embolism Prevention,

2. Durability,
3. Ease and Security of Attachment,
4. Preservation of Surrounding Tissue Function,
5. Reduction of Turbulence
6. Reduction of Blood Trauma
7. Reduction of noise
8. Use of Materials Compatible with Blood and Tissue,
9. Development of Methods and Sterilization

[Matthews]

The materials used in a mechanical heart valve are selected primarily for their non-thrombogenecity and or high strength. During early heart valve development some of these materials like Silicone or Teflon were selected primarily for their bio-inert ability. However, as time has passed people realize that these materials although not thrombogenic, do cause infections and or develop scar tissue, which ultimately can end in thrombosis. Table 2.2 details commonly used materials in mechanical heart valves.

Table 2.2 Materials used in Mechanical Heart Valves

Disk materials	Sewing ring	Cage	Poppet
Silicone rubber	Dacron	Titanium	PTFE (Halon)
Delrin	Teflon	Haynes 25	
Silastic	Titanium	Stellite alloy no 21	
Graphite Coated Metal	Polyester knit fabric		
Pyrolytic carbon	UHMWPE with embedded Titanium ring		
	Polypropylene cloth		

2.4 Proposed design

As previously mentioned in the introduction, the new proposed design is to construct a heart valve from a bio-degradable scaffold. The scaffold after being seeded with cells would be placed inside a bioreactor for the growth period. To develop Acellular tissue, a scaffold is needed, this scaffold provides a foundation for the cells. After a sufficient quantity of cells have been attached to the scaffold, a process begins whereby the ratio of polymer scaffold to endothelial cells decreases, in the end leaving only cells. During the formation, deposition, and organization of the newly generated matrix, the scaffold is either degraded or metabolized, eventually leaving a vital organ or tissue in place. During this physical shift, it is necessary that the scaffold provide the strength, and elasticity, needed during the timeframe where the model is mostly scaffold. It is believed, that cells that are created during this time period, need to be exposed to process parameters, similar to those seen in the tissue being duplicated. This process equivalency will result in the organs being closer in terms of physical characteristics to their natural component. "By seeding cells onto a porous valve like scaffold and subjecting the seeded scaffold construct to conditions in the incubator similar to those inside the heart, the researchers were able to produce functional tissue engineered heart valves." [Applied Genetic News] Also, "Recent work with cartilage and blood vessels indicates that applied shear stress has a beneficial effect on the quality and quantity of the generated tissue. Blood vessels grown with a well defined shear stress showed a higher content of myosin heavy chains, a higher burst strength, higher collagen content, improved

suture retention, and better morphologic appearance than vessels obtained under non-pulsed culturing." [Stock] Not only must the scaffold material have the necessary physical characteristics to promote proper cellular growth, but it also must be biocompatible and "meet both nutritional and biological needs for the specific cell population involved in tissue formation." [Stock]

The cells used in any tissue engineering model need to be native to the organ that is being mimicked, the native cell for the heart valve is the endothelial cell. Endothelial cells are the same type of cell that comprises arteries and veins, although they do not have the same physical properties, e.g. burst strength, wall thickness. Studies are currently being done to determine if these sites can be used for replication purposes. Since a direct biopsy for either a heart valve or main artery is not practical, an alternate site must be selected, and in this case, a peripheral vein may be acceptable. It has also been seen where dermal fibroblasts have been used during cellular replication. "The cellular component of normal heart valve tissue is composed mainly of endothelial cells and myofibroblasts. Therefore, tissue for cell harvesting and in vitro expanding can be obtained from vascular structures that contain similar cell components. Based on this consideration, we used human aortic myofibroblast in the present study." [Zund] Another option is to use Stem cells, these cells have the ability to rapidly proliferate and differentiate into a wide variety of cells found in the human body. Once the cells have been harvested and placed in the growth medium, a certain amount of knowledge must be ascertained with regards to the control of differentiation and cellular proliferation. If the cellular growth is not controlled

during this process, then the valve will not have the correct physical appearance and its physical characteristics will not be representative of a native valve. Unlike many of the organs found in the body, heart valves do not need a micro-vascular system to obtain their nutrients and rid themselves of their waste. Heart valves receive their nutrients by way of diffusion, which avoids a major technical obstacle that faces many tissue engineers today, the creation of a micro-vascular system. Any scaffold material selected for this project would need to satisfy a variety of criteria's, which are detailed below:

1. Biocompatibility
2. Biodegradable, controlled erosion process
3. High cellular attachment strength
4. Physical properties, i.e. elasticity, tensile strength similar to heart valves

Work has already been done in creating scaffold materials that could fill these characteristics; they have been used in either heart valve or vascular tissue engineering projects. Dr. Simon Hoerstrup and his colleagues of the Harvard Medical School are working diligently on developing a Tissue Engineered Heart Valve. This group has developed a unique process whereby they can create a three dimensional heart valve scaffold, via a Stereolithography melt forming process. The material that this group has chosen for their scaffold is a poly-4-hydroxybutyrate (P4HB) (Tepha Inc., Cambridge, Ma), "P4HB is a semicrystalline, thermoplastic elastomer with a melting point of approximately 60C and a glass transition temperature of –51C." [Sodian] This material has worked very well, "The heart valves were tested in a pulsatile bioreactor, and it

was noted that the leaflets opened an closed synchronously under sub-physiological and supra-physiological flow conditions." [Sodian] Since the proposed design is structured around the concept of a biodegrading material then an explanation of the degradation cycle of PLA may be needed. "Water penetrates the bulk of the material, usually attacking the chemical bonds in the amorphous phase and converting long polymer chains into shorter water soluble fragments. Because this occurs in the amorphous phase initially, there is a reduction in molecular weight without a loss in physical properties, since the device matrix is still held together by the crystalline regions. The reduction in molecular weight is soon followed by a reduction in physical properties as water begins to fragment the device. In the second phase, enzymatic attack and metabolization of the fragments occurs, resulting in a rapid loss of polymer mass. This type of degradation when the rate at which water penetrates the device exceeds that at which the polymer is converted into water-soluble materials (resulting in erosion throughout the device) is called bulk erosion. All of the commercially available synthetic devices degrade by bulk erosion." [Applied Genetics News]

- Polyglycolic-acid (PGA)

The first synthetic absorbable suture, it was the first bio-polymer used in the successful creation of a new tissue, it is a highly crystalline material, with a high melting point. PGA looses about 50% of its strength after two weeks and 100% after four weeks it is completely absorbed in 4 – 6 months.

- Polylactid-acid (PLA)

Poly(dl-lactide) DLPLA is an amorphous material, it has a low tensile strength high elongation and a rapid degradation time, used frequently as a drug delivery system.

- PGLA

A 90/10 copolymer of PGA and PLA, has been used successfully in a heart valve leaflet substitution experiment.

- Hydrogels

Hydrogels are used mostly in drug delivery devices, change physical states from a liquid to a gel during implantation.

- Poly-3-hydoxybutyrate and Poly-4-hydoxybutyrate

Poly-4-hydoxybutyrate has a high elasticity and the ability to control its degradation rate, not much is known about Poly-3-hydoxybutyrate however its physical properties should be fairly close to Poly-4-hydoxybutyrate.

- Polyhydroxyoctanoate (PHO)

Presently this material is being developed by Tepha Inc. and studied by Dr. Ralf Sodian's of the Harvard Medical School. It is biodegradable and believed to have good elastic properties.

- PHA4400

A strong and flexible material, has been successfully used as a bio-absorbable material, the material used during Dr. Simon Hoerstrup's heart valve experiments.

Ideally, one of the materials listed above could be used in this project, the scaffold design could be simply an exact duplicate of a human heart valve. The

valve would perhaps have a sewing ring attached to it, which would give the bioreactor a place to hold the valve during growth and also give the surgeon an area to suture during surgery. During the growth process the bioreactor pressure gradient and flow rate would need to be investigated as well as the materials rate of degradation and the amount of growth media used. Thus in conclusion, the ideal tissue engineered heart valve would be comprised of the patient's own cells, have physical properties similar to a native Heart valve and have a short Bioreactor growth period.

Table 2.3 Tissue Engineering Scaffold Materials

Material	Tensile Modulus	Ultimate Tensile Strength	% Elongation
PLA	290,000 psi	9860 psi	5
Polyamide	478,000 psi	8000 psi	100
Polycaprolactone	63,000 psi	3600 psi	800
PGA	1,015,264 – 2,030,528 psi	110,229 – 133,435 psi	18 – 25
PLGA (Vicryl)	1,015,264 – 2,030,528 psi	82,671 – 131,984 psi	18 – 25
Artery	4.35 – 435 psi	145 – 232 psi	0.8 – 1.1
Heart Valve	5947 – 9282 psi	290 – 653 psi	10 – 18
Elastic Cartilage	2176 psi	435 psi	30
Skin	3336 – 6382 psi	899 – 2031 psi	110 – 140
Tendon	140,107 psi	8557 – 10,008 psi	8 – 9

2.5 Conclusions from literature review

People are living longer these days, unlike 50 years ago it is not uncommon to see many people live beyond their 70'th birthday. Coinciding with this extended life span is a desire by many people to be able to live the life that they did when they were 50 while they are 70. This cannot be done with the biomedical

prosthesis available today, the devices available today only have a ten to fifteen year lifespan. A large majority of people begin having major surgery when they reach their mid to late 50's, so by the time they reach their mid 70's their medical prosthesis are at the end of their lifespan. Surgery to remove these types of devices is almost impossible, taking into consideration their age and their debilitating health condition. Therefore, a change needs to be made if society is going to reach the next plateau in life expectancy. Permanent organ and tissue replacements need to be developed that will perform equivalent to the original tissue or organ being replaced. The proposal being recommended in this thesis is just such a device, if successful could meet all of these requirements.

There are many issues that need to be addressed to develop a Tissue Engineered Heart Valve, such as: selecting the correct material, determining the correct Bioreactor process conditions, controlled tissue growth, achieving high cellular attachment strength, uniform dimensional geometry of the scaffold, developing the scaffold manufacturing process, and many others.

In the literature review, the proposed design discusses the finished product being a representative physiologically valve, for this thesis however the author will devote his time to the selection of a material, the processing of the samples, and the subsequent physical testing. There will be no cellular growth development with these scaffolds or degradation studies. If this thesis was brought any further beyond this initial work, those area's and others would be investigated thoroughly. The next step to be taken is to develop a test protocol, which will detail the material to be used, and a testing procedure. The materials

selected for this study will most likely be selected from the list of materials detailed in table 2.3. The test procedure will be an attempt at developing a test format to be used to characterize the physical properties of the scaffold materials. Therefore, in conclusion the test sample design for this study will be a flapping hinge design instead of an actual heart valve.

CHAPTER 3

RESEARCH OBJECTIVE

For the remainder of this thesis, the body of the report will deal with the work that went into the development of the scaffold manufacturing process, the final process used in making the samples, and the testing that was done to fully characterize the scaffolds. The material selected for the scaffold development is Poly-Lactic-Acid (material samples provided by Johnson & Johnson), this material was selected for its ability to meet several of the design requirements listed in the previous sections. Poly-Lactic Acid (PLA), is a biodegradable polymer that has the processing flexibility needed to achieve satisfactory results. Other materials mentioned in the literature review may have lower tensile strength properties and higher ductility, possibly making them a more appropriate candidate, however those companies did not make their materials available for this study.

Since PLA does not have the flexibility needed for a tissue scaffold, modifications were made to the material in an attempt to increase its flexibility. Compounding Bovine Collagen with PLA as well as creating a porous structure using NaCl was used. The high ductility of collagen when combined with the PLA will translate to a lower overall tensile strength. NaCl, was added to create a porous structure in the scaffold, this would accomplish two things, one decrease the tensile strength of the scaffold and secondly create sites available for cellular attachment.

CHAPTER 4

MATERIAL DEVELOPMENT

4.1 Process development

During the early parts of the research, it was discovered that there are two ways of processing PLA, the polymer chosen as the material to be used in this study. Those two ways are solvent casting and heat forming, the latter was attempted early on, however, it became rapidly apparent that it would be difficult and costly to develop. Using the heat forming method would remove process flexibility from the material compounding process. Therefore, the solvent casting was selected for the following reasons, it is easy to modify, inexpensive to produce samples, and lastly, it offers flexibility during the compounding process.

Once the decision was made to process the scaffold samples via solvent casting, a series of rapid experiments were conducted to determine the optimum parameters and methods. The mold designed to make the initial prototype samples was a three dimensional model that was made to resemble a working valve. Potential solvents were examined to determine which solvent would most aggressively dissolve PLA, i.e. alcohol, Hexane, Methanol, and eventually Methylene Chloride, which was found to work the best.

During this initial period prior to making the final scaffold samples, an enormous amount of information was gathered about the intracies of processing. These areas will be discussed in more detail below, however for now they are

listed as: Scaffold Geometry, Scaffold Homogeneity, Solvent evaporation, and Preparation of the PLA Gel.

4.2 Scaffold geometry

The geometry of the scaffold both the thickness and the profile are dependent on processing conditions and illustrated clearly in the test results. The initial prototype sample, shown below in figure 2.5 was designed to imitate a valve, not an actual heart valve, but a two way leaflet valve. It was difficult to control the geometry of this valve due to the overall thickness of the valve and the varying rate of solvent evaporation. During the evaporation period the solvent will evaporate from the scaffold in some areas faster than in others, thus causing large voids to occur in the scaffold. Also, due to the solvent being present during this period, there is a large amount of shrinkage that needs to be taken into consideration. During the time that the three dimensional mold was being used, a hurdle was reached, after the PLA gel was injected into the mold, how would it be separated without damaging the geometry of the part? Introducing a step whereby after the gel had been injected

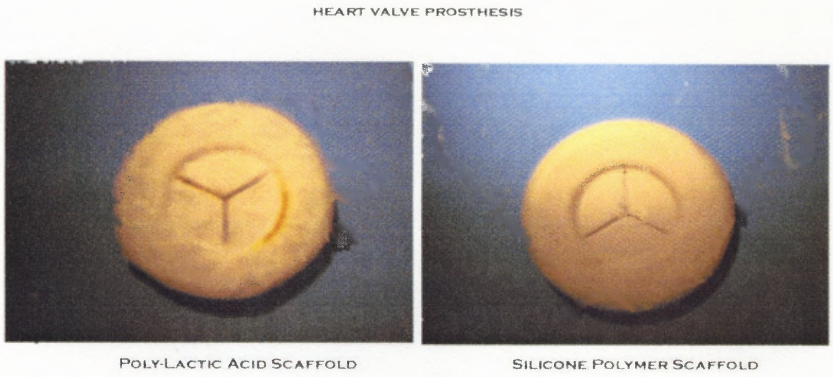

Figure 2.5 Early heart valve scaffolds made with the 3D process method, PLA material on the left and Silicone on the right.

into the mold, it was immersed in a liquid Nitrogen bath to freeze the Methylene Chloride solved this, after several minutes at –190F the mold sample is easily removed. The sample is then placed in a environmental hood for solvent evaporation. After the sample has been removed from the environmental hood it is removed from the mold, due to the significant difference in thermal expansion between steel and the PLA scaffold it is easily removed from the mold.

4.3 Scaffold homogeneity

The most desirable characteristic of each valve scaffold would be reproducible scaffold samples with uniform cross sections, absent of voids or foreign matter. The voids are created due to air bubbles created during the PLA gel preparation period, during the solvent evaporation period sections would collapse and leave craters in their place. Objects that may be present in the scaffold could be either foreign matter captured during the processing period or fragments of PLA resin, not fully dissolved during the solvent dissolution period.

4.4 Solvent evaporation

Once the PLA resin has been combined with the Methylene Chloride, mixed thoroughly and formed into the desired shape it must be placed into a vacuum oven in order to remove the solvent. For a valve to be made using this process method, the solvent must be entirely removed from the scaffold. TGA testing has been done to determine the proper oven temperature, and drying time, to be

used to fully remove the solvent from the samples. The results from that testing will be discussed in full detail in the results section.

4.5 Preparation of the PLA gel

In the beginning of the PLA solvent dissolution development the resin was simply added with solvent and mixed thoroughly, and then more solvent was added to the compound until the resin was deemed to be in gel form. This process takes time and consumes a large amount of solvent. A preliminary step was added to the process whereby the resin is crushed, until it is in the form of a fine powder, thus increasing the PLA surface area and increasing the rate of dissolution. Now by using the PLA in a powder form during the solvent process, takes a fraction of the time, and reduces the amount of solvent needed significantly.

4.6 Solvent injection molding

After several attempts to mold scaffold samples, the process was found to work well, after the top plate of the mold was removed, the exposed sample looked acceptable. However, over time the sample would deform and the top portion of the scaffold would change dimensionally. At this point it was determined that to construct a useful three dimensional scaffold, the thickness of the scaffold must be at a minimum. The top section of the thickened scaffold, since it was exposed to the air, would loose solvent first and then voids left by the solvent would collapse and form an uneven landscape on the top portion of the valve. Detailed

below is a description of the procedure that was used during the initial scaffold preparation for use with the three dimensional mold.

1. The resin is weighed out to the specified amount and placed in a beaker.

2. The specified amount of solvent (Methylene Chloride) is added to the beaker.

3. The resin/solvent solution is covered while inside of the beaker and allowed to sit for several hours to allow the resin to fully dissolve in the solvent.

4. The gel (resin/solvent) is mixed aggressively until it is homogenous.

5. The gel is poured from the beaker into a 2 oz. Syringe, evacuating the air from the syringe and forcing the gel to the Luer tip, a tip cap should be used to cover the luer tip.

6. The 2-piece mold is assembled and the screws holding the mold together are hand tightened, (if the screws are tightened too much they will become very difficult to be removed after the freezing step).

7. The syringe luer is inserted into the mold entry port and the gel is injected into the mold by actuating the plunger rod.

8. After the mold has been filled with the solution, it can then be immersed in the liquid Nitrogen bath for several minutes.

9. Once the mold has been removed from the liquid Nitrogen bath it should be immediately separated (protective gloves should be worn when removing the mold from the bath) thus keeping the sample in one piece.

10. Place the sample, present in only one portion of the mold into the environmental hood and let sit for 24 hours to let the solvent evaporate.

11. Remove the sample from the mold once it has been totally dried of the solvent.

4.7 Solvent cast molding

Taking the lessons learned from the initial mold, a second mold was created; this mold was much simpler and designed to create thinner prototypes that could enable the molded material to be more flexible (same modulus less volume). Another lesson learned was that instead of just one prototype made from one batch of solution, the new mold had five cavities so an entire group of samples can be made and tested from one batch. Similarly, as was done with the initial polymer process, resin will be dissolved in Methylene Chloride. However, there is no need to inject into a mold, these samples will be simply poured into their individual cavities and evenly dispersed throughout the cavity. With the injection molding process samples were produced that accurately portray a valve. However, to learn more about the materials, the casting method can be used. The new solvent casting method is detailed below:

1. The resin is weighed out to the specified amount and placed in a beaker.
2. The specified amount of solvent (Methylene Chloride) is added to the beaker.
3. The resin/solvent solution is covered while inside of the beaker and allowed to sit for several hours to allow the resin to fully dissolve in the solvent.
4. The gel (resin/solvent) is mixed aggressively until it is homogenous.

5. The gel is poured from the beaker into a 2 oz. Syringe, evacuating the air from the syringe and forcing the gel to the Luer tip, a tip cap should be used to cover the luer tip.

6. Using the syringe, the material is injected onto the cavity, and then the material is rolled into the cavity, whereby flattening the material and filling up the cavity at the same time.

7. Once the material has been formed into all of the cavities, the plate is transferred to the drying oven for the solvent evaporation period.

8. After 4 hours of drying the samples in the oven, it can be removed and placed into their identification bags.

CHAPTER 5

DESIGN ANALYSIS AND TEST PROTOCOL

As mentioned in the previous pages, the ideal heart valve must be as strong and flexible as a natural heart valve, and degrade in a controlled manner while allowing steady cellular attachment with strong adhesion. Two separate concepts have been incorporated into this study, in a attempt to meet two of the previously mentioned ideal scaffold characteristics. Those two characteristics are controlled flexibility for extended periods of time, and increased cellular attachment strength. The two concepts being investigated in this thesis are; Developing a porous structure via a Sodium Chloride leaching, and the compounding of Bovine Collagen with the PLA to increase flexibility and fatigue life.

5.1 Scaffold porosity and degradation rate

The polymer to pore ratio would be varied during any further investigation, low, medium, and high distributions. Preliminary studies, have already given some insight into the effect of pores on the polymers ability to degrade and physical characteristics during the degradation period. Studies have shown that PLA and PGA materials degrade via Bulk degradation method, also that as the amount of pores are increased in the polymer that the degradation time will increase. The physical strength of the polymer will decrease as the amount of pores is increased. Recent studies have shown that polymers exposed to flow during the cellular attachment period, rather than static conditions culturing process results

in a longer degradation time period. "The hydrolysis of PLA-PGA materials is catalyzed by the presence of a high concentration of carboxylic end-groups; thus the degradation products of PLA-PGA materials serve as catalysts for the reaction." Therefore if these degradation products are not removed from the bioreactor during operation then the breakdown of the polymer will be accelerated. "Thus, the mass, molecular weight and elastic modulus data clearly indicate that PLA-PGA scaffolds degrade faster when they are less porous/permeable and when they are not subjected to fluid flow." [Agrawal] With regards to polymers exposed to cyclic flow another variable that must be investigated during this process is whether the fluid is turbulent or laminar while it is in contact with the scaffold. The state of the fluid (turbulent or laminar) will almost certainly have an effect on the degradation kinetics of the material as well as the cells during their attachment process.

Previous research has shown that pore distribution as well as size will have a dramatic effect on both the ductility and degradation time of the scaffold. The environment inside of the bioreactor will necessitate a ductile material that can oscillate repeatedly, however the degradation time needs to be minimal, such that cell growth can replace the scaffold without causing a bulking of the composite (polymer scaffold/cellular tissue).

As the scaffold is oscillating under a specified pressure and flow inside the bioreactor the endothelial cells will be attaching themselves to the surface. Recent work has shown that the pressure and flow that the endothelial cells are exposed to during this attachment process will dictate the strength and

orientation of the attached cells. To aid in the attachment process of the endothelial cells a porous structure will be created in the valve. The distribution and concentration of the pores and their size will be crucial for the cellular attachment period since unlike with collagen synthetic polymers do not have natural binding sites.

The most widely used natural polymer is collagen and the most widely used synthetic polymer is PGA, PLA or PLGA. The characteristics being investigated are the modulus of elasticity and the flexural strength. These components of the material will dictate how successful they will be during the cyclic loading. The modulus of elasticity testing will relate how well the material will oscillate, and the flexural strength will determine the force needed to oscillate the valve.

5.2 Protein and cellular seeding process

The process of the endothelial cells attaching themselves to the biodegradable polymer is an intricate process whereby to be successful several areas need to be properly investigated. These areas include the following; growth factors, scaffold pore structure, protein and cellular seeding, and bioreactor design and operation. "Research suggests that the initial adhesion of smooth muscle cells to these polymers is mediated by both fibronectin and vitronectin binding, but over time (24hr), cellular adhesion is primarily mediated by binding to vitronectin." [Nikolovski] Vitronectin is the primary protein seen with synthetic polymers, however both fibronectin and vitronectin have been observed to attach

themselves to natural polymers (collagen). The determining factor as to the effectiveness of the completed valve, will be determined by the cellular orientation and strength of the scaffold.

Cellular attachment strength is dependent upon the following protein seeding, polymer pore structure, biomaterial surface activity and biological growth factors. Synthetic polymers do not have natural binding sites therefore protein attachment is vital to the overall process since they will provide the only attachment points for the cells. During the growth cycle cellular orientation is primarily due to the flow cycle seen in the Bioreactor. Prior to cellular seeding the scaffolds should be bathed in an extra cellular matrix with selected proteins present, these proteins will adsorb to the surface and provide the necessary attachment junctions. Once proteins have adsorbed and the cells have attached themselves to the proteins (integrins attach themselves to the proteins and link themselves to the endothelial cells) growth factors will be introduced to accelerate the growth cycle.

5.3 Test protocol

5.3.1 Background

In the beginning of the thesis a introduction to the heart valve device field was given, including a discussion of the evolution of the mechanical heart valve, as well as devices both mechanical & tissue currently used in the market. Then the goal of this research was stated, a biodegradable polymer used for a heart valve scaffold. The next sections covered were the background information on the

development of the scaffold manufacturing process, as well the introduction to the chosen material, PLA. Therefore now that the heart valve field has been introduced, the materials have been discussed, and the process has been fully explained, it is time to discuss the next to last section, testing. The test protocol being discussed below is a testing regimen the author believes will help in characterizing the chemical and physical properties that are vital to a successfully operating heart valve scaffold.

5.3.2 Objective:

The objective of this protocol is to determine through physical and chemistry testing the physical characteristics of each material. These tests are being used to identify the best possible candidate to be used for a tissue engineered heart valve scaffold.

5.3.3 Test Supplies and Equipment

1. Poly-L-Lactide, PURAC, biochem
2. Bovine Fibrillar Collagen, Datascope, Collagen Products Division
3. Methylene Chloride, Sigma
4. Ethylene Glycol, Sigma
5. Sodium Chloride, + 80 mesh, ALDRICH
6. 50ml Glass Beaker, Kimax
7. Aluminum Scaffold Casting Plate
8. 2 oz. Plastic Syringe, Becton Dickinson
9. 2 inch wide Parafilm, American National Can

10. Stabil Therm Electric Vacuum Oven, BLUE M, General Signal
11. Stainless Steel Stirring Spoon
12. Instron test stand, Tensile/Compression model # 5564

5.3.4 Sample Description

Table 5.1 – Test Sample Description	
Group identification	Processing conditions
Group A [4g] Poly-Lactic-Acid [8ml] Methylene Chloride	Samples will be dried in vacuum oven between 35 – 40C Samples removed from the oven at: 0.5, 1.5, 2.5, & 4hrs
Group B [4g] Poly-Lactic-Acid [12ml] Methylene Chloride	Samples will be dried in vacuum oven between 35 – 40C Samples removed from the oven at: 0.5, 1.5, 2.5, & 4hrs
Group C [1g] Bovine Collagen [10ml] Methylene Chloride	Samples will be dried in vacuum oven between 35 – 40C Samples removed from oven after 2 hrs
Group D [4g] Poly-Lactic-Acid [8ml] Methylene Chloride Poured over bed of 200um NaCl crystals	Samples will be dried in vacuum oven between 35 – 40C Samples removed from oven after 4 hours Rinsed with Water for 4 hours to remove NaCl
Group E [5g] Poly-Lactic-Acid [10ml] Methylene Chloride [0.8g] Bovine Collagen per scaffold Ethylene Glycol	Samples will be dried in vacuum oven between 55 – 60C Samples will be removed from oven after 19 hours

5.3.5 Procedure

Four out of the five scaffold materials, detailed above in Table 5.1, will be made using the solvent casting method detailed previously in section 4.7. One of the

objectives of this study is to determine the amount of solvent needed to fully dissolve Poly-Lactic-Acid.

5.3.6 Test descriptions

1. Flexural testing, to determine the force necessary to bend the scaffold as it would be done in a bioreactor.

2. Cycle testing, To obtain qualitative visualization of the scaffold in operation. It needs to be determined whether the material in question can successfully operate without fracturing during operation, and if fracturing does occur how many cycles. Scaffold samples will be immersed in a heated solution during the testing. The scaffold will be held rigidly at one end while a load is being applied repeatedly to the other end of the scaffold. The load will be applied at a rate of 60in/min with a total displacement of 1in, for 60 cycles.

3. Tensile testing, to determine the tensile strength of the material in question. Test samples will be uniformly prepared in a simple rectangular shape, such that they can be easily tested in a tensile testing machine. The samples will be inserted into the machine such that both ends are held securely at both ends. Upon activation the machine will pull the sample in tension to its breakage point, at a rate of 1in/min.

4. FTIR, (Fourier Transform Infrared Spectroscopy), to determine the chemical composition of the material in question.

5. SEM, to photograph the material under high magnification to characterize the pore structure and overall architecture of each material.

6. TGA, (Thermo gravimetric Analyzer) measures the change in mass as a function of temperature under a controlled atmosphere. The TGA can be used to analyze de-absorption and decomposition behavior and characterize oxidation behavior. The TGA may be used to characterize phenomena such as evaporation and drying, decomposition, oxidation, and oxidative stability. Its principal uses include measurement of a material's thermal stability and composition.

7. DSC, (Differential Scanning Calorimeter) measures the amount of energy (heat) absorbed or released by a sample as it is heated, cooled, or held at a constant temperature. Typical applications include determination of melting point temperature and the heat of melting; measurement of the glass transition temperature; curing and crystallization studies; and identification of phase transformations.

5.4 Physical test results

Table 5.2 Test Sample Tensile Testing Results

Group	Displacement at peak (in)	Load at Peak (lbf)	Stress at Peak (psi)	Displacement at Break (in)	Load at Break (lbf)	Stress at Break (psi)	Young's Modulus (Ksi)
A	0.034	45.492	1449.990	0.044	20.866	665.070	27.565
A	0.027	41.655	1632.413	0.042	15.327	600.642	39.381
A	0.027	35.797	1088.697	0.040	12.964	394.274	23.907
C	0.095	5.706	91.299	0.162	2.021	32.341	6.203
C	0.077	4.134	82.674	0.227	1.488	29.753	5.635
C	0.124	3.282	65.631	0.227	1.130	22.610	2.910
D	0.020	30.292	651.434	0.020	30.292	651.434	21.990
D	0.022	37.954	888.437	0.022	37.954	888.437	24.715
D	0.025	40.779	846.890	0.025	40.779	846.890	23.244
E	0.042	40.095	1336	0.042	40.095	1336.5	50.268
E	0.047	29.275	975.825	0.058	10.895	363.167	44.395
E	0.045	55.385	1846.16	0.05	31.648	1054.93	97.034
Heart Valve	Unk.	Unk.	471.5	Unk.	Unk.	Unk.	7614.5

Table 5.3 Scaffold Flexural Test Results

Group	Maximum Bending Force (lbf)	Load at 0.25" (lbf)	Load at 0.5" (lbf)
A	0.049	0.036	0.049
A	0.143	0.099	0.143
A	0.057	0.044	0.056
C	0.008	0.007	0.004
C	0.014	0.014	0.006
C	0.012	0.009	0.010
D	0.164	0.119	0.164
D	0.226	0.166	0.225
D	0.153	0.108	0.153
E	0.210	0.150	0.210
E	0.147	0.088	0.147
E	0.330	0.234	0.330

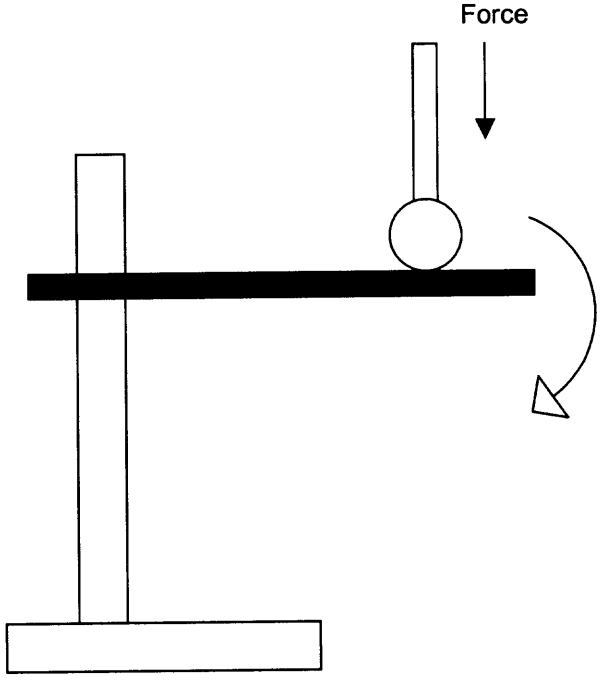

Figure 5.1 Diagram of the scaffold flex testing setup.

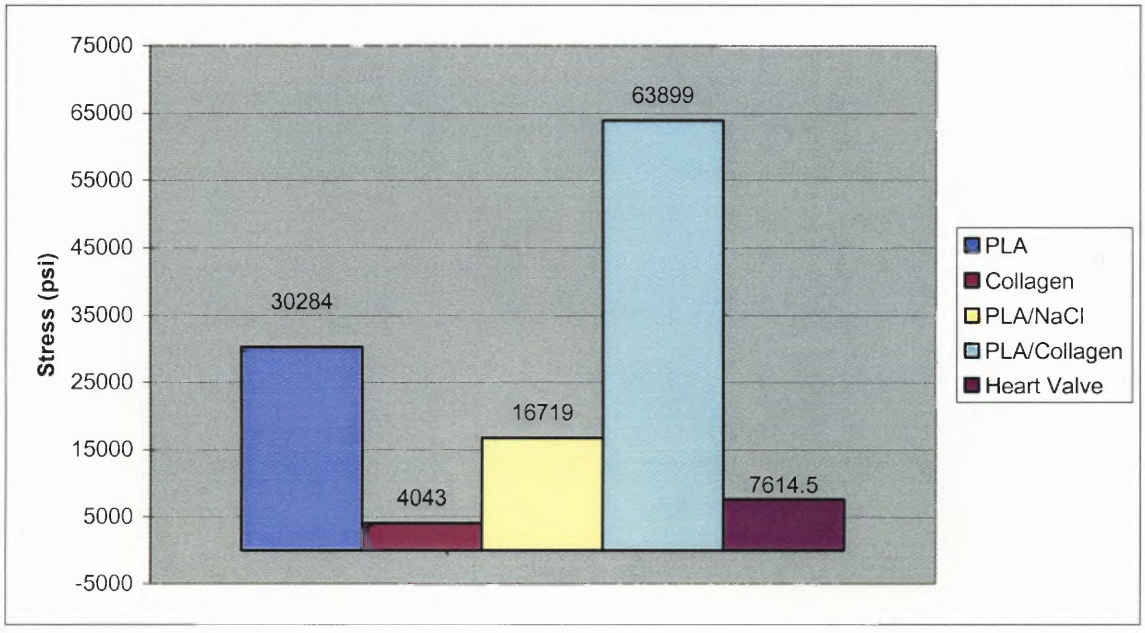

Figure 5.2 Young's Modulus comparison of Scaffold materials, values given are averages.

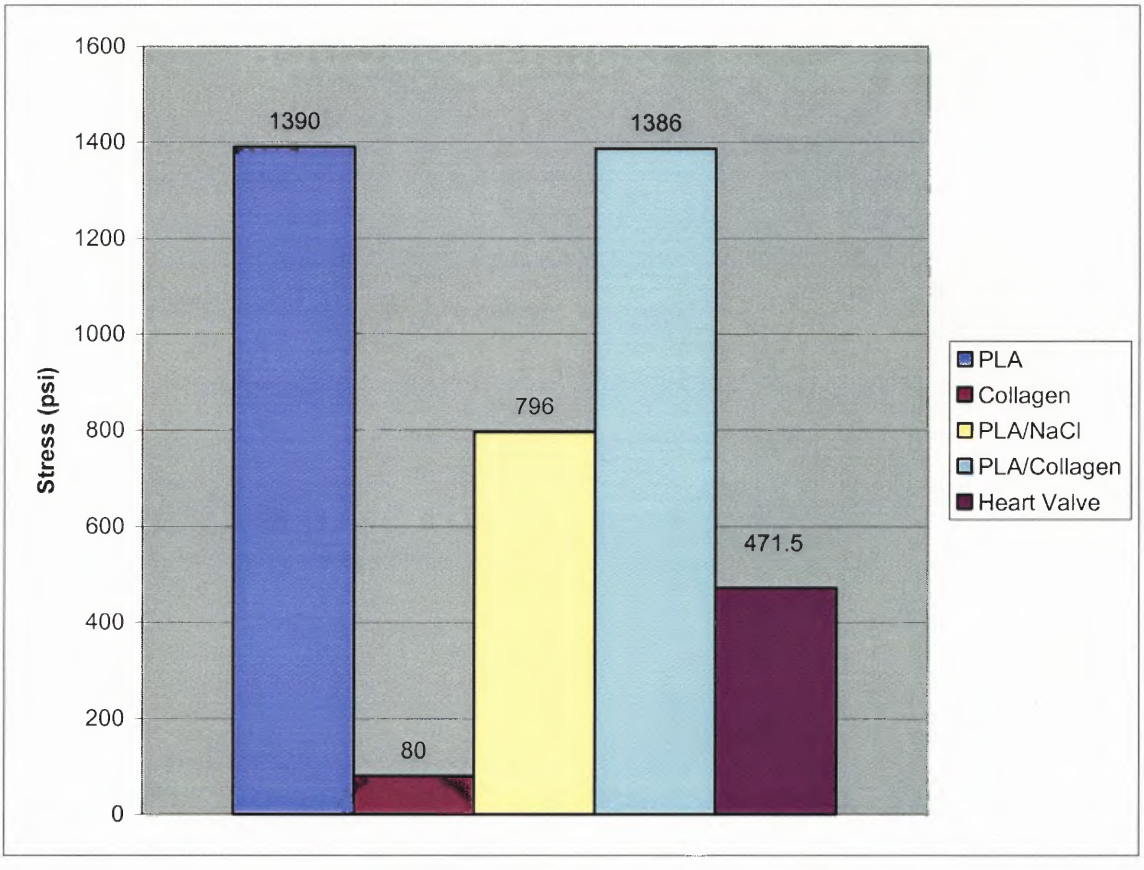

Figure 5.3 Ultimate Tensile Strength comparison of Scaffold materials, values given are averages.

5.5 Discussion of results

At the beginning of this study, the goal set forth was to identify a material that could be used as a biodegradable scaffold for the use in a Heart Valve Bioreactor. The physical and chemical results obtained through testing, illustrates the need, for further development with these materials. Using Poly-Lactic-Acid and Bovine Collagen simultaneously offers several benefits, optimal physical characteristics as well as superior cellular attachment strength – a characteristic not tested in this thesis.

5.5.1 Tensile testing

The tensile test results are located in table 5.2, the testing was conducted on a Instron test stand and the samples (dry and at room temperature) were pulled in tension until failure at a rate of 1"/min. The Ultimate Tensile Strength (UTS) of the PLA, both groups A and E are much greater than that of a Heart Valve's UTS, although they have a much lower % elongation. The fortunate case with the PLA having such a high UTS is, that when the thickness of the Scaffold drops even lower than previously tested, it will still be able to function adequately. The UTS of the PLA/NaCl scaffold seems to have been effected from the porous structure created by the NaCl, its tensile strength and Young's Modulus are similar to the Heart Valve omitting the elongation property. When combined with the PLA, the collagen did show to have an effect on the Tensile Strength, therefore, while adding cellular recognition sites, the collagen when combined with PLA can provide needed flexibility.

5.5.2 Scaffold Flex Testing

The Scaffold Flex test results are located in table 5.3, the testing was conducted on a Instron test stand, (testing apparatus is detailed in figure 5.1) the samples (dry and at room temperature) were deflected at a rate of 10"/min for a distance of 0.5in. The bending force was on the high side for nearly all three groups, exception being the Bovine Collagen group. A problem seen with the Collagen group was, that the reaction force to the bending force was not seen to be substantive. The collagen samples were very weak and offered very little resistive force when deflected. For a scaffold to be successful this reaction force, must be inherent in the material such that, after the scaffold has been flexed it will quickly recover to its original position.

5.5.3 Valve Cycle Testing

The cycle test results are located in appendix A; the samples were immersed in heated water and tested in the same apparatus as in the flex testing. The load was applied to the scaffold at a rate of 60in/min, and the load was applied for a distance of one inch. Therefore, since it took one second to displace the valve, and one second to return to its resting place, the total cycle test lasted for two seconds. The scaffold cycle in this thesis operated at a rate of 30 cycles/minute, a normal heart valve will operate at a rate of 75 cycles/minute, therefore, more testing is needed at a higher cycle rate.

Assuming a physiological pressure at the valve of 140mm/Hg, and a cross sectional area of 1.169in^2 (diameter based on a 31mm St. Jude Bi-Leaflet prosthesis) then the force applied to open the valve during the cardiac cycle will

be roughly 3.168lbs. Cycle testing would need to be done as a benchmark on actual heart valves, to determine the range of force seen during normal operation. This force cannot be too high or it would restrict blood flow, too low and the valve will not stay closed while the ventricle is filling. During the cycle testing, the scaffold showed no signs of fracturing or stress. The collagen on the PLA did however, begin to separate from the PLA while in the warm solution. The PLA when exposed to the cycle testing performed adequately, even though the samples were not at their optimum thickness, or appearance. The cycle testing done in this thesis, is a preliminary feasibility study, and was focused on determining the ability of the scaffold to cycle, over a short time period. More cycle testing would need to be done, for longer time periods, at higher cycling rates, and under bio-relevant conditions.

5.5.4 TGA testing

The TGA test results are located in Appendix B, there are eleven TGA graphs attached in this appendix. The objective of conducting TGA tests on the material was, to determine if the solvent used during the manufacturing process stayed in the scaffold, after 0.5, 1.5, 2.5, and 4.0 hours of drying in a vacuum oven, at an elevated temperature (60C). The table shown below, listed as Table 5.4 lists the temperatures for selected points on a TGA curve for the three groups (A, B, & Control) which represent three different PLA/solvent ratios, (50%, 30%, and 0%). The points identified in figure 5.3 represent distinct points, in the curve that characterize specific areas in the TGA test cycle.

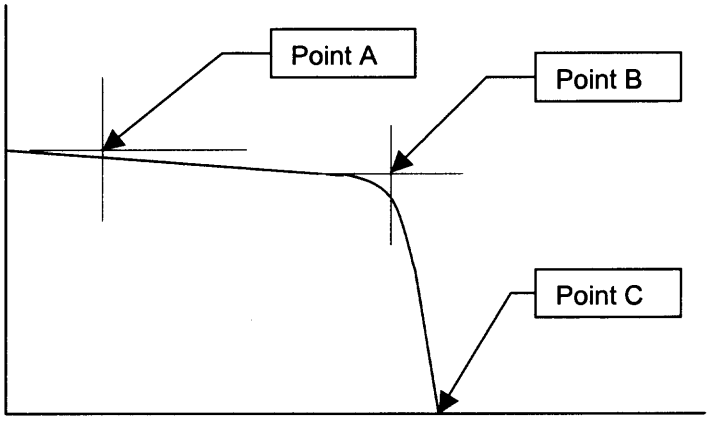

Figure 5.4 TGA test curve description, critical points identified.

Table 5.4 TGA Testing of PLA samples

Group A – PLA Scaffolds – 50% Weight/Volume ratio				
	Dried for 0.5 hrs.	Dried for 1.5 hrs.	Dried for 2.5 hrs.	Dried for 4.0 hrs.
Point A	127°C	150°C	165°C	130°C
Point B	337°C	337°C	337°C	337°C
Point C	402°C	405°C	402°C	402°C
Group B – PLA Scaffolds – 50% Weight/Volume ratio				
Point A	130°C	125°C	125°C	135°C
Point B	335°C	335°C	335°C	335°C
Point C	400°C	400°C	405°C	410°C
Un-Adulterated PLA Resin – No Solvent or Drying				
Point A	300°C			
Point B	330°C			
Point C	420°C			

The TGA testing of the PLA samples (Group A & B) revealed that the solvent still resides within the material. The point C for groups A & B was fairly consistent with each other, but quite different from the un-adulterated resin. This shows that the amount of solvent used during the manufacturing process is inconsequential. Regardless of the amount of solvent used during the process, both groups showed to have roughly 1.5 – 2.0% of the material being solvent. The drying process did not seem to have an effect on the material; there was no significant difference between 4 hrs. of drying and 1.5hrs. of drying. The curve for

the PLA resin is very smooth and shows no scatter during the test, the same cannot be said of groups A & B. After point B the curve for groups A and B bounces up and down for a short time period, and then eventually straighten out. As the material reaches 100°C the material begins to melt, which is occurring before the melting point of PLA. Both these characteristics are evident in both groups A & B and are evidence of the methylene chloride boiling out of the PLA, during the TGA test cycle.

5.5.5 DSC testing

The DSC test results are located in Appendix C, there are three graphs attached in Appendix C, which are Unadulterated PLA, PLA/Collagen, and PLA/NaCl. The results obtained during the DSC testing, come to the same conclusion reached during the TGA testing, which is, solvent still resides in the scaffold. The solvent seems to be removed from the material roughly in the temperature range of 100 – 150C. The bimodal peaks in the PLA/Collagen and PLA/NaCl curves represent a reorganization of the crystalline structure during the heating process. Both PLA scaffold materials tested, showed a low level of crystalline regions in the polymer structure.

5.5.6 FTIR & SEM testing

The FTIR test results located in Appendix D were done to identify the chemical structure of the scaffolds. The FTIR curves for the following groups: PLA, PLA/NaCl, & PLA/Collagen had almost identical peaks. There was however, an increased amount of small absorbance scatter seen in the PLA/NaCl and

PLA/Collagen samples between 4000 – 2000cm^{-1} wavenumbers, as compared to the control. The pure Collagen group had a more unique curve which shows a large and wide peak around 3700 – 2700 wavenumbers, this peak although small is visible with the PLA/Collagen group. The PLA/NaCl group is nearly identical to the pure PLA group, indicating that there was little to no NaCl left over from the processing procedure.

The SEM picture located in Appendix E is a high magnification picture of the PLA/Collagen scaffold surface. As hoped during the design of the materials the scaffold has large peaks and valleys, which during the cellular growth process would provide excellent adhesion points. The next step would be to increase the distribution and orientation of the collagen throughout the scaffold. The PLA/NaCl sample was unable to be identified under the SEM, however, further development of the porous structure, including increased pore size and quantity is needed.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS

6.1 Issues with present material

The samples made for this Thesis were shown to have adequate physical properties, high physical strength but low elongation. It is believed that for a scaffold to be successful it must behave similarly to a more ductile material, e.g. Latex, Silicone. Assuming a heart rate of 75 beats/minute, then an average heart valve will cycle 108,000 times in one day. Currently the only material that can withstand that workload for as long as say fifty years is an individual's own heart valve tissue. This material can last that long because it is constantly regenerating itself, therefore, the requirement of the scaffold studied in this thesis is too last long enough for the living tissue to take over.

The PLA samples made during this study were done using a solvent, it was found from TGA and DSC testing 1.5 – 2.0% of the material is methylene chloride. This last amount of solvent still present in the material may prove to be very difficult to remove, in fact the only way to decrease or remove it may be in reducing the thickness of the scaffold as previously discussed. As was seen in the DSC testing, the morphology of the material is not uniform, which is undesirable, random crystalline segments reside within the polymer. Therefore, while the test results look promising and many obstacles have been overcome, there are two major obstacles that need to be surpassed. The first obstacle is to determine, whether or not the scaffold can survive the cycle load, long enough

for it to be replaced by living tissue, if not, then what modifications to the material can be made to enable it to survive. The second obstacle, is to determine if the solvent can be removed from the material, perhaps it can be accomplished with a thinner scaffold dried over a longer time period at lower temperatures. Whatever the method is, the solvent must be removed from the material, a material cannot be used as a tissue scaffold in a medical procedure if it contains methylene chloride. The author believes that these two issues are the primary obstacles facing any further development of PLA as a heart valve tissue scaffold, however, several methods mentioned in this thesis, and listed again below, can be used to solve these design issues.

6.2 Solutions to issues

Detailed below are possible solutions to the design issues mentioned in the previous section, which again are improving the flexibility of the scaffold, and removing solvent from the material.

1. Improve the geometry of the scaffold samples. By better controlling and improving the manufacturing process the cross section will be more uniform throughout the sample. Another improvement to the geometry would be to further decrease the thickness, possibly in the range of 0.010 – 0.020inches.
2. Further develop the scaffold compounding process. The collagen and NaCl seem to have had a beneficial effect on the physical strength of the scaffold. It is already known from literature sources, that collagen is a

beneficial component during the cellular attachment and orientation process. The amount of NaCl used in the study can be increased and the process should be developed further. These two components, collagen and NaCl, can both increase the flexibility of the material while also helping during the growth process.

3. Improve the post Scaffold molding process. After the samples have been molded they are placed into a vacuum oven and raised to an elevated temperature. This drying process, used to eliminate the solvent from the scaffold, has not worked. More process development is needed here to determine, if this last remaining amount of solvent can be removed from the material. Options that may be successful could be any of the following: increased drying times, bathing in water post processing, change of solvent, introduction of heat during the molding process, and finally, further reduction in the scaffold thickness.

6.3 Possible future development

In this Thesis the author has touched upon three key areas for any successful Tissue Engineering Scaffold, porosity, flexibility, and dimensionally accurate. Test results collected from this research has shown, that using a porous PLA in combination with collagen can provide to be a successful heart valve scaffold. Preliminary testing has been helpful however; a more detailed setup for both the flex and cycle testing is needed. The tests need to be refined in their handling of the samples, as well as incorporating bio-relevant attributes to them.

The solvent injection molding process used early in the process development needs to be re-investigated. The solvent injection process can create three dimensional scaffolds, which are necessary for any further scaffold development. With the advancements made during the process development cycle such as, refinement of the resin, reduction in solvent, and incorporating thinner, more uniform cross sections, it is possible that the solvent injection molding process may be used.

As the material is being further developed as mentioned previously, work must begin on the cellular growth process. There needs to be a study done to determine the cellular attachment strength and orientation with the PLA scaffolds, as well as extreme cycle testing to determine their fracture point. Therefore a bioreactor would need to be designed to facilitate in the development of the scaffold material. This bioreactor would need to provide the pressures, and flow rates found in the chamber of the Heart, in order to grow endothelial cells in the proper manner. A Bioreactor design being offered by the author is detailed in Appendix F.

In conclusion, the author believes that the possibility of developing a biodegradable polymeric scaffold for a Heart Valve is great. The advancements made in this thesis, have shown that it is possible to use PLA for just such a purpose. More work is needed to develop a heart valve scaffold, however, the groundwork has been established and a target has been set. It is the author's belief that if the development outlined in this thesis is continued, then a living heart valve can, and will be created.

APPENDIX A

SCAFFOLD CYCLE TEST RESULTS

Cycle testing of the PLA/Collagen scaffold, sample was immersed in warm solution and deflected 60 times over a two minute period.

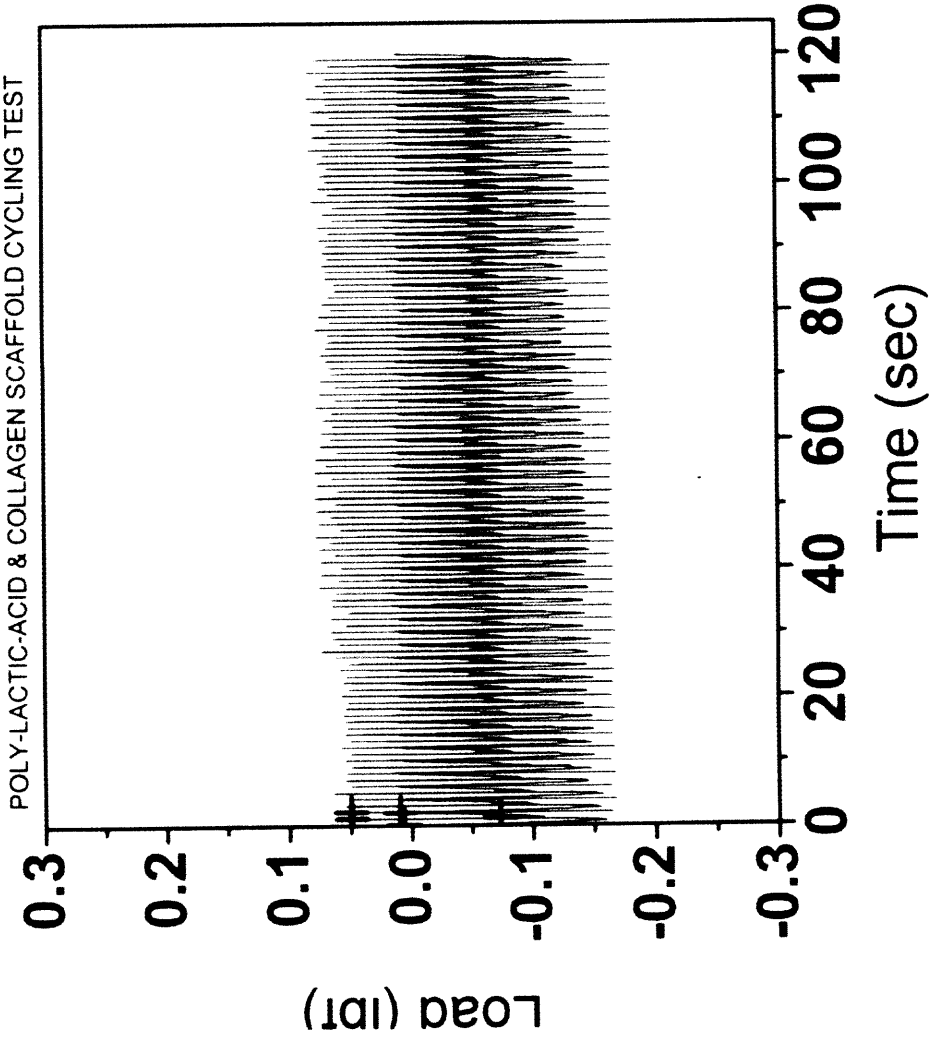

Figure A.1 Valve cycling of PLA/Collagen scaffold, 120 second scale.

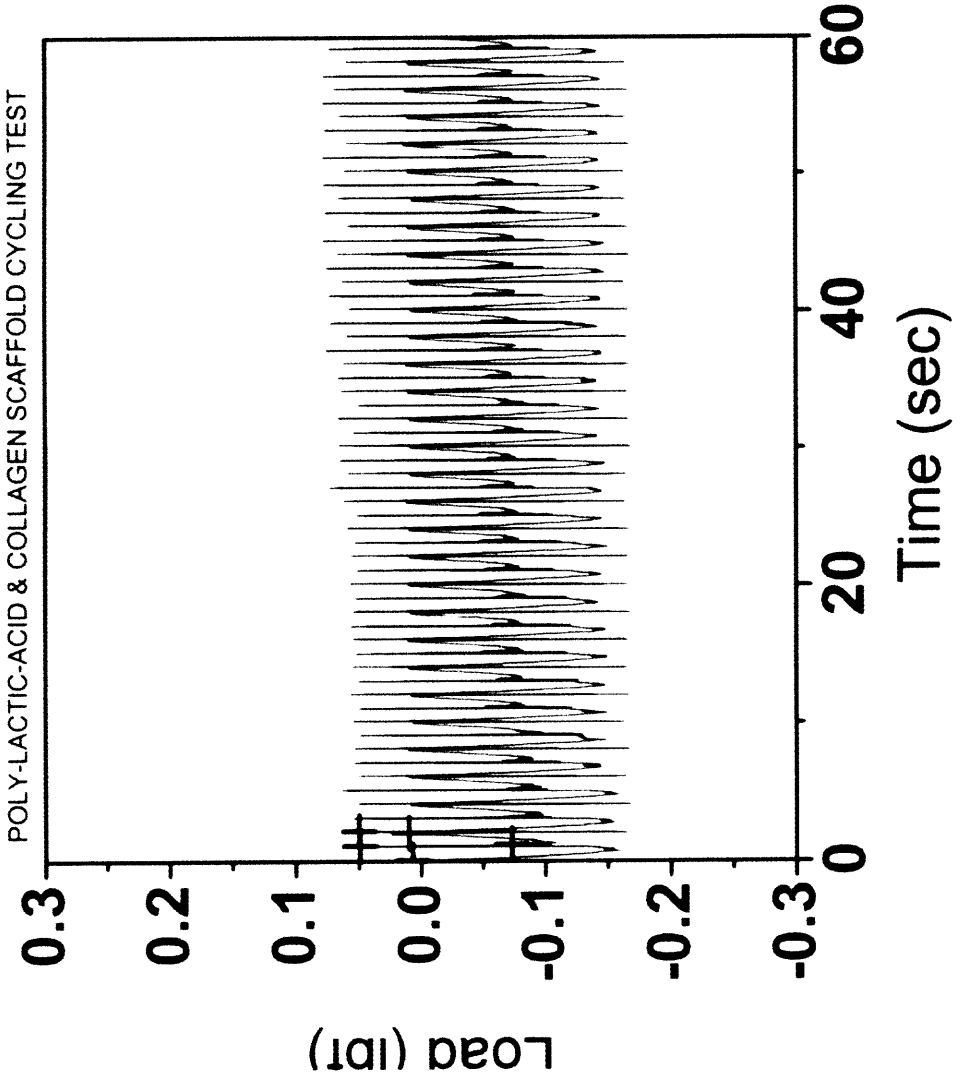

Figure A.2 Valve cycling of PLA/Collagen scaffold, 60 second scale.

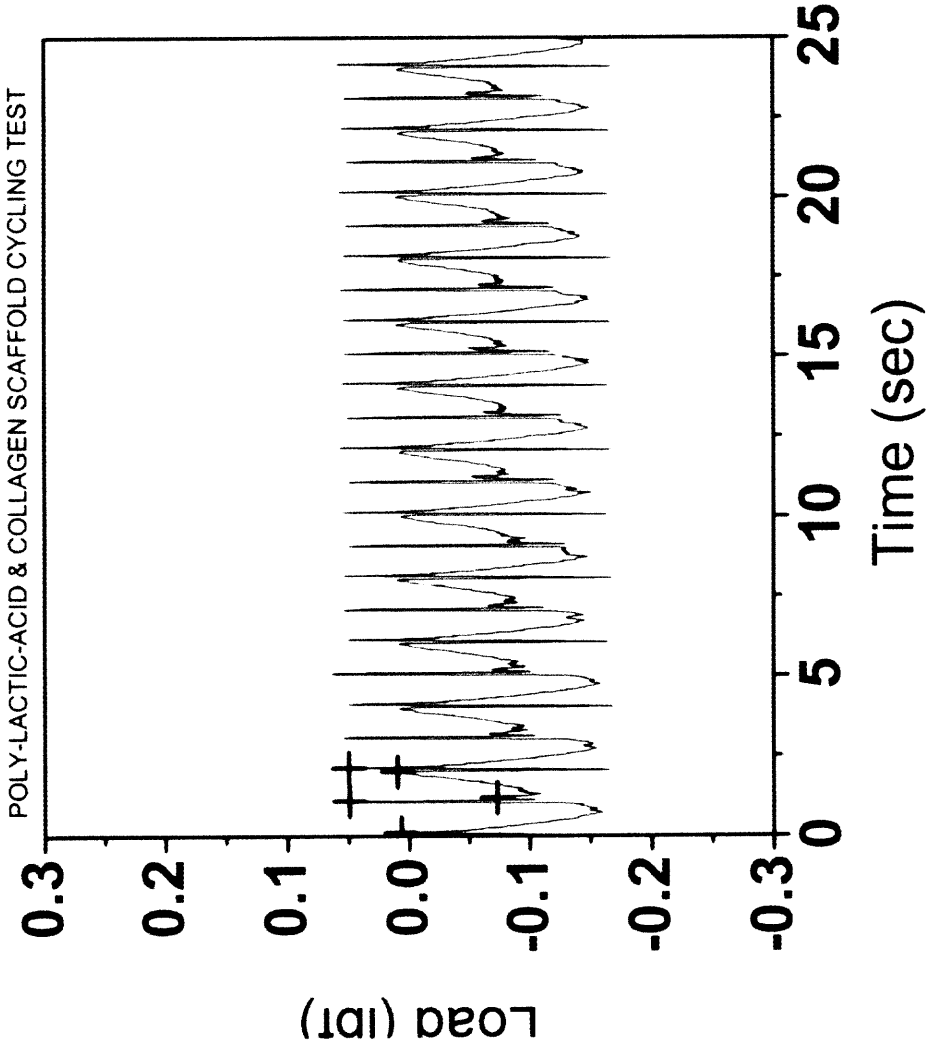

Figure A.3 Valve cycling of PLA/Collagen scaffold, 25 second scale.

APPENDIX B

TGA TEST RESULTS

TGA testing of PLA scaffold samples, this testing was done to determine the amount of solvent still present in the material after the casting operation.

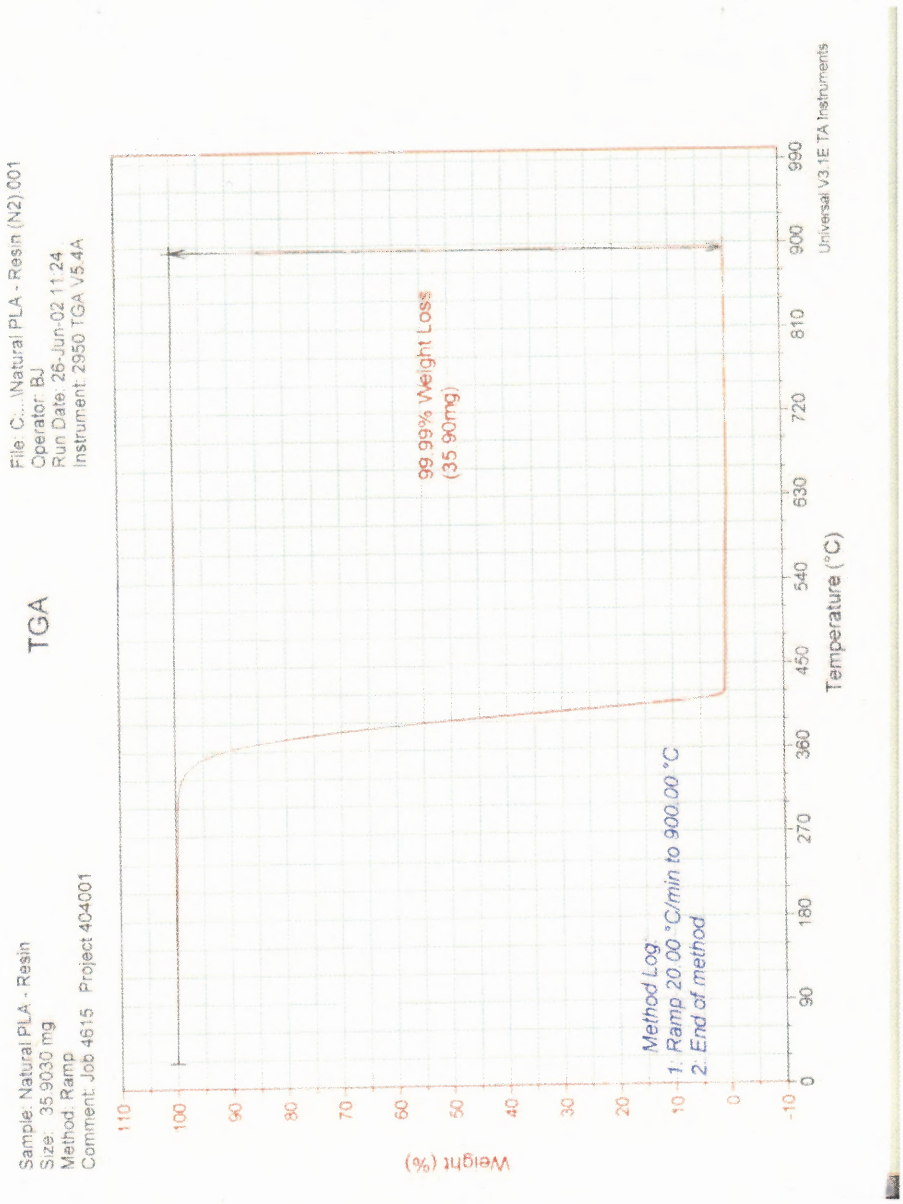

Figure B. 1 TGA testing of natural PLA resin.

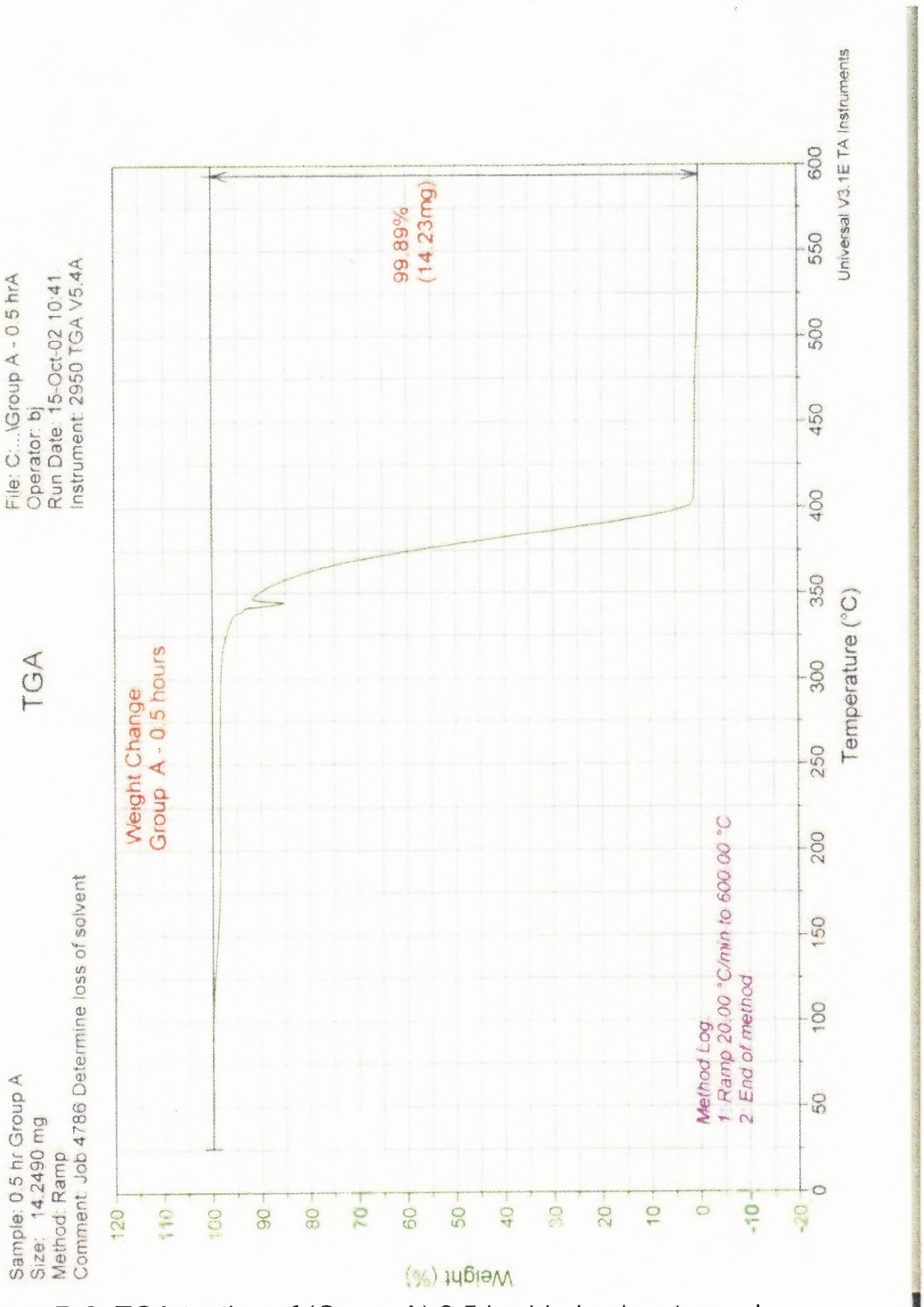

Figure B.2 TGA testing of (Group A) 0.5 hr dried solvent cured PLA –scaffold.

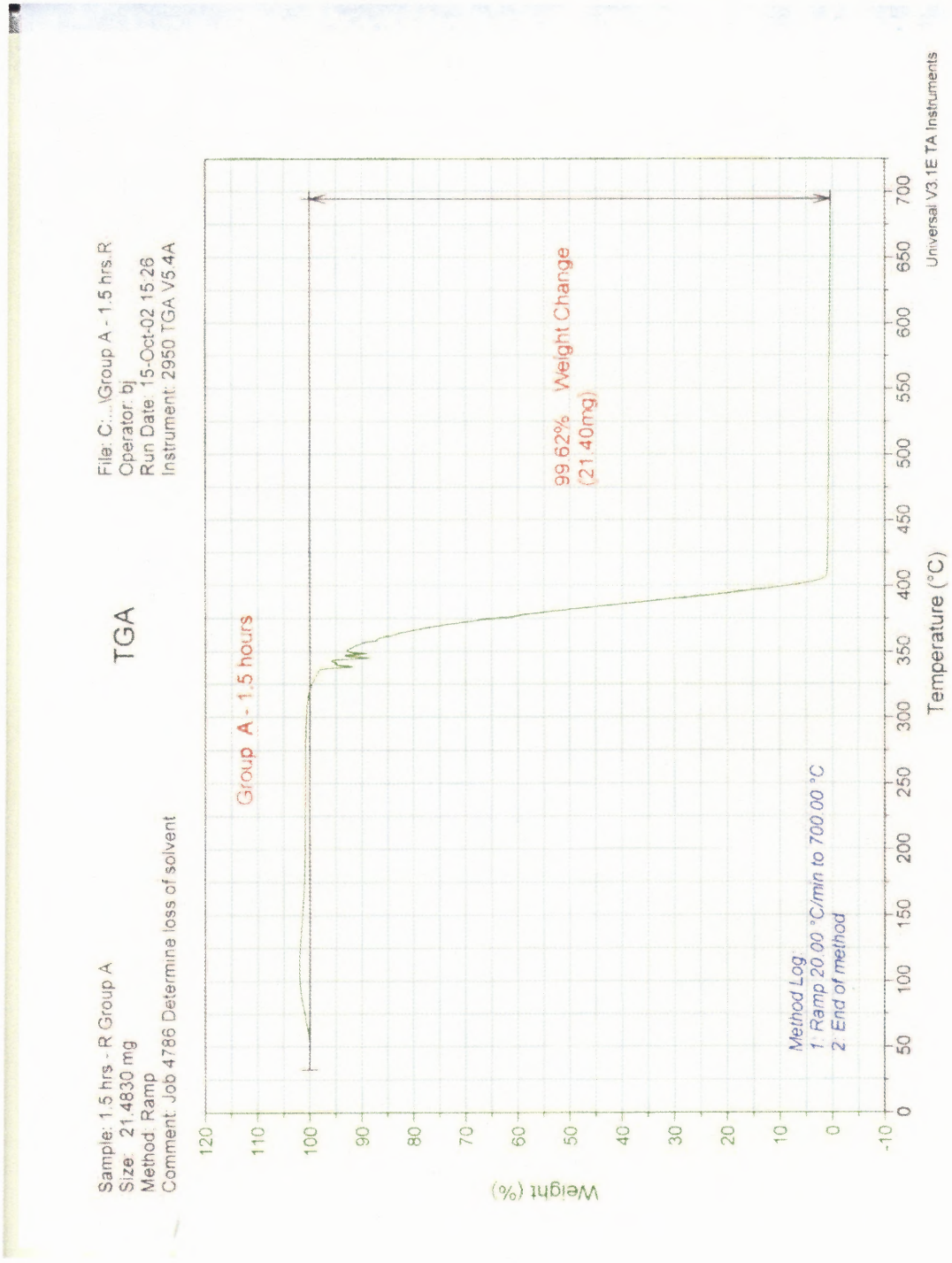

Figure B.3 TGA testing of (Group A) 1.5 hr dried solvent cured PLA –scaffold.

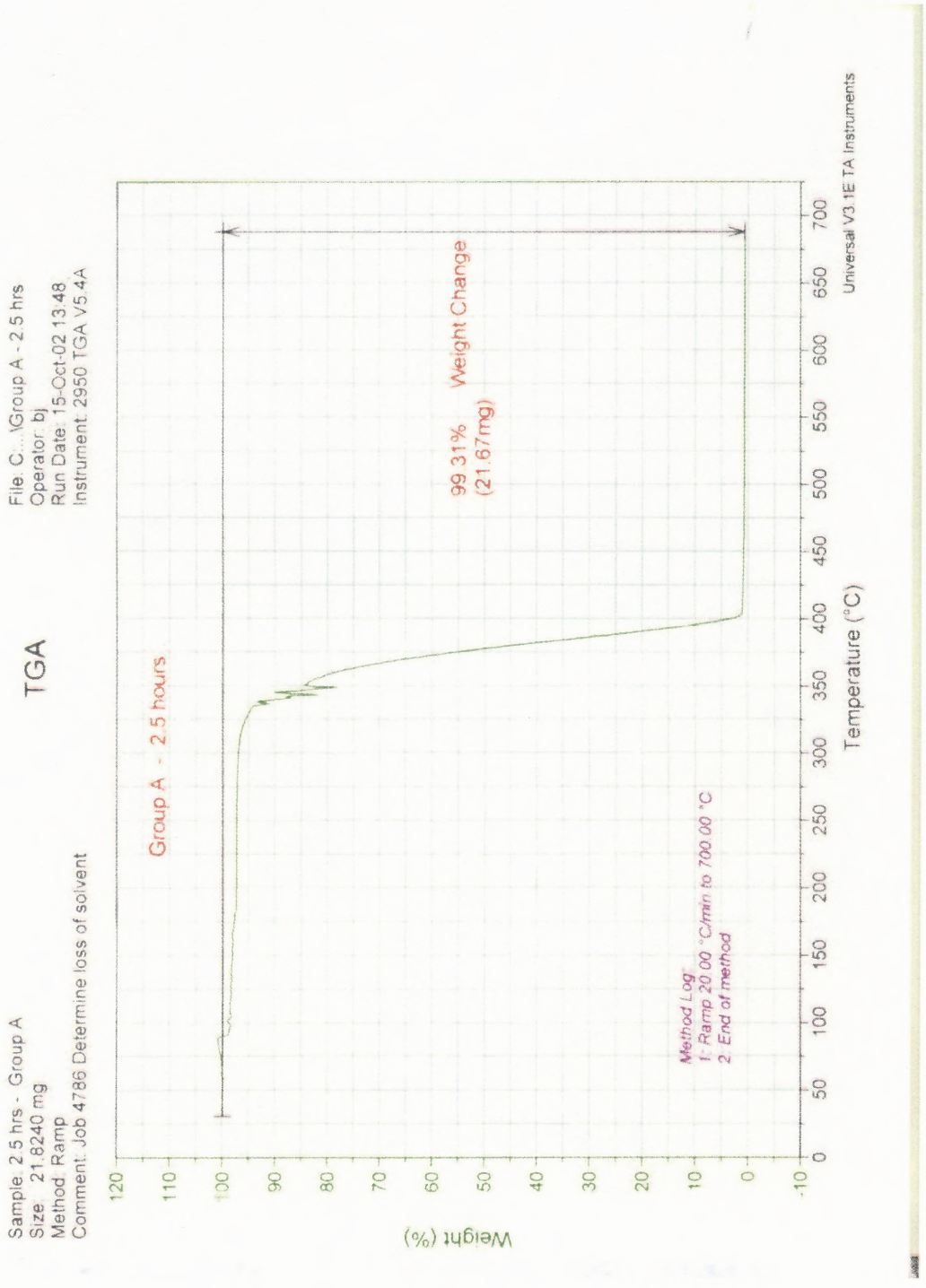

Figure B.4 TGA testing of (Group A) 2.5 hr dried solvent cured PLA –scaffold.

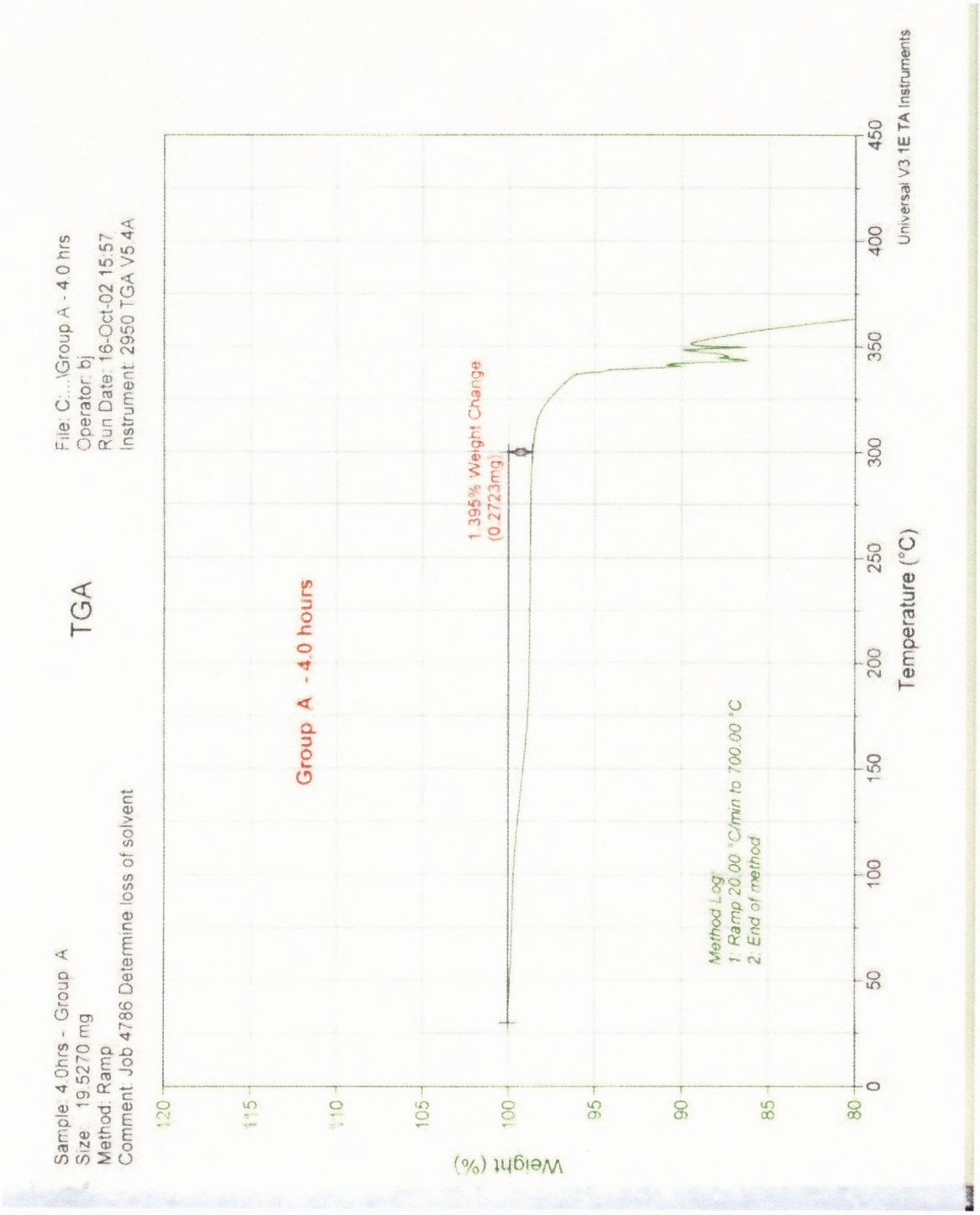

Figure B.5 TGA testing of (Group A) 4.0 hr dried solvent cured PLA –scaffold.

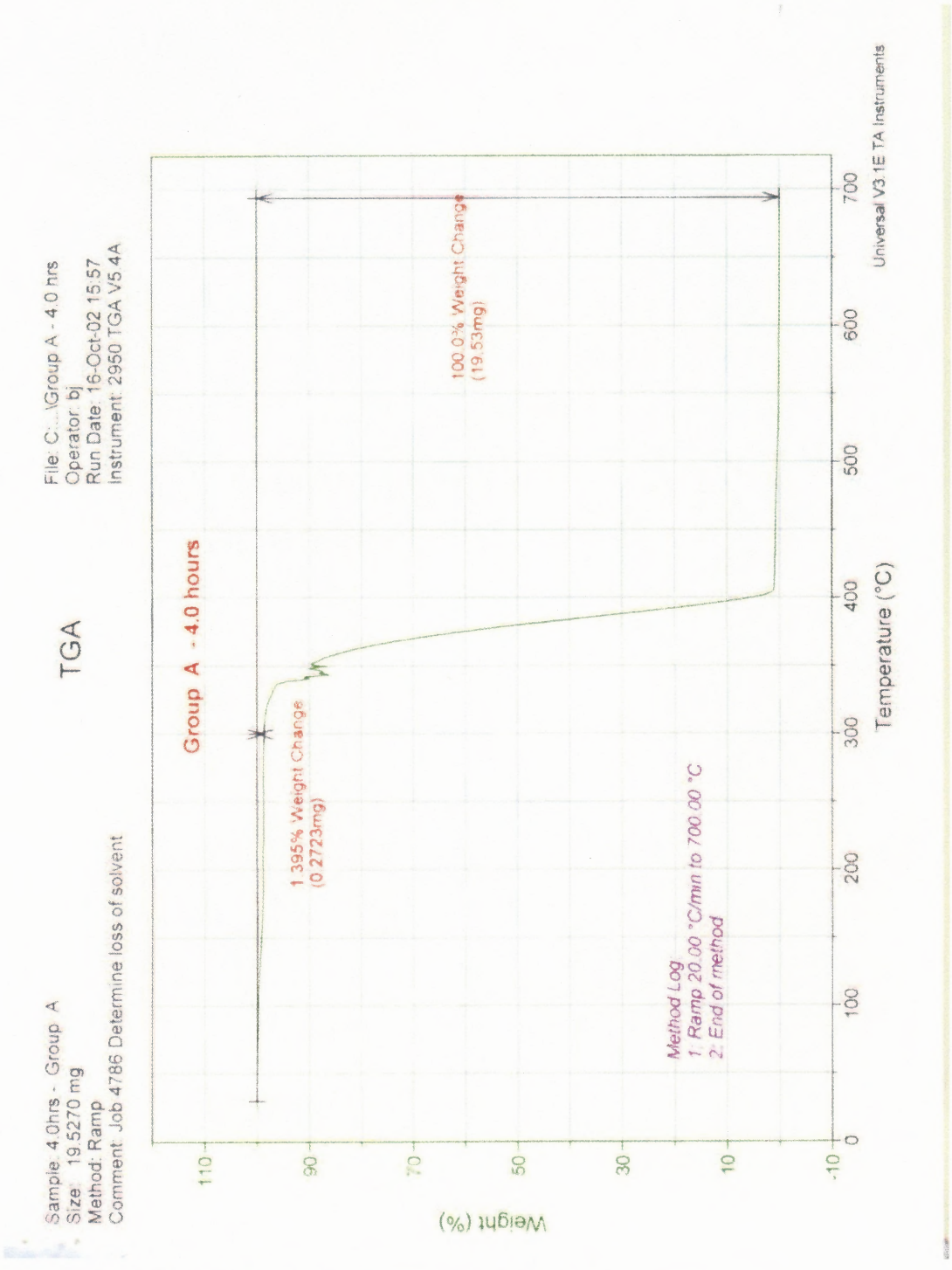

Figure B.6 TGA testing of (Group A) 4.0 hr dried solvent cured PLA –scaffold.

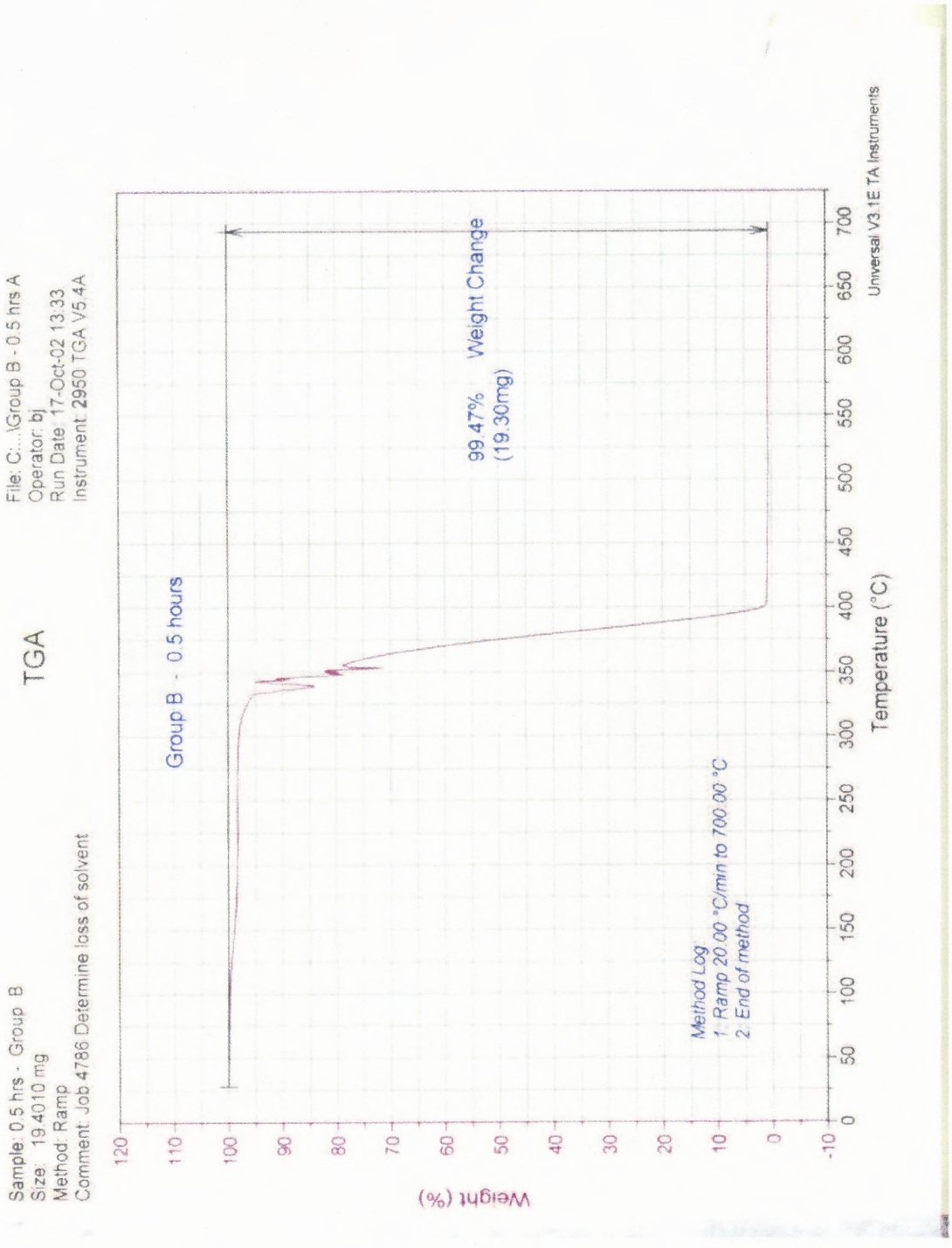

Figure B. 7 TGA testing of (Group A) 0.5 hr dried solvent cured PLA –scaffold.

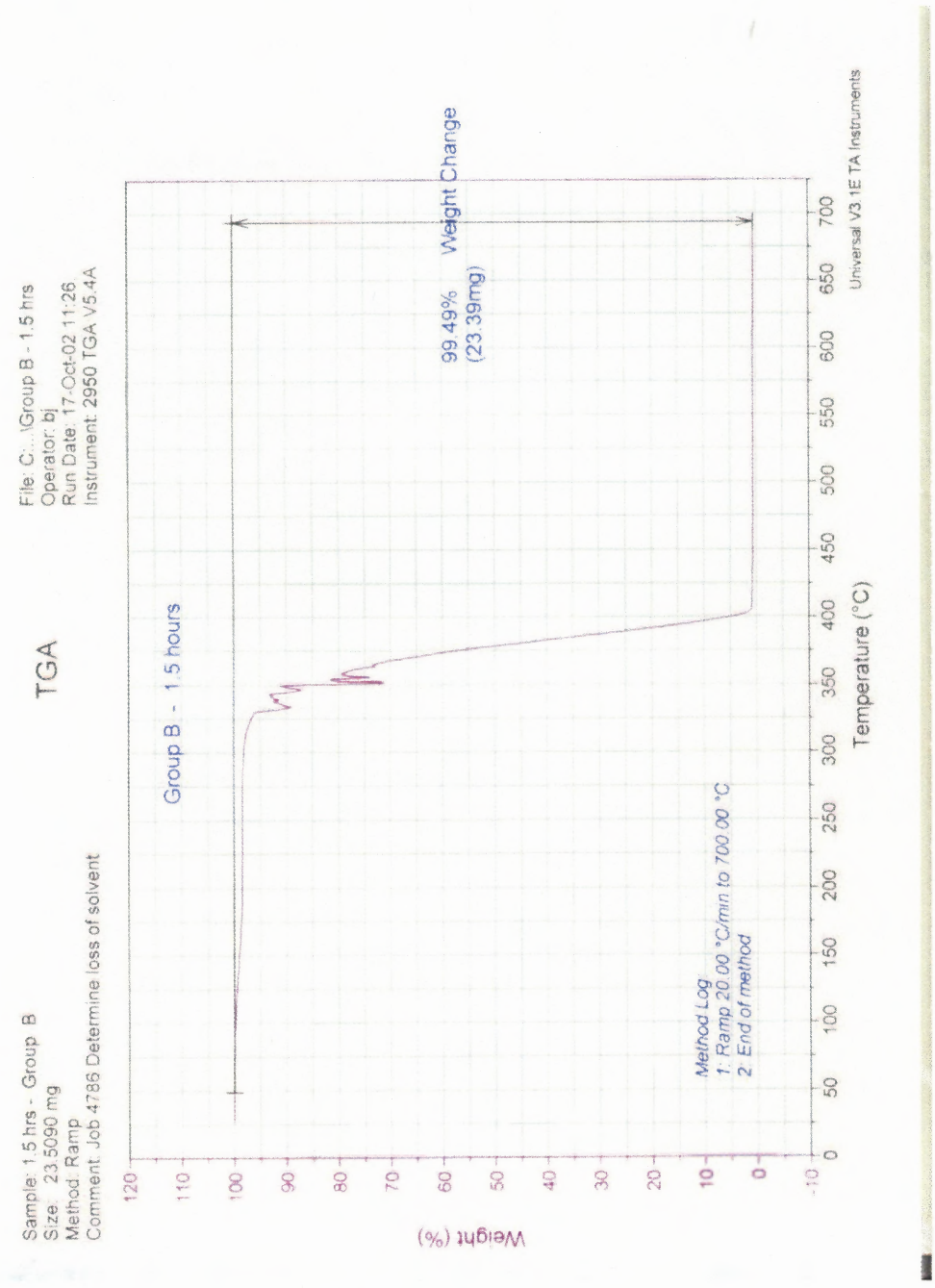

Figure B. 8 TGA testing of (Group B) 0.5 hr dried solvent cured PLA –scaffold.

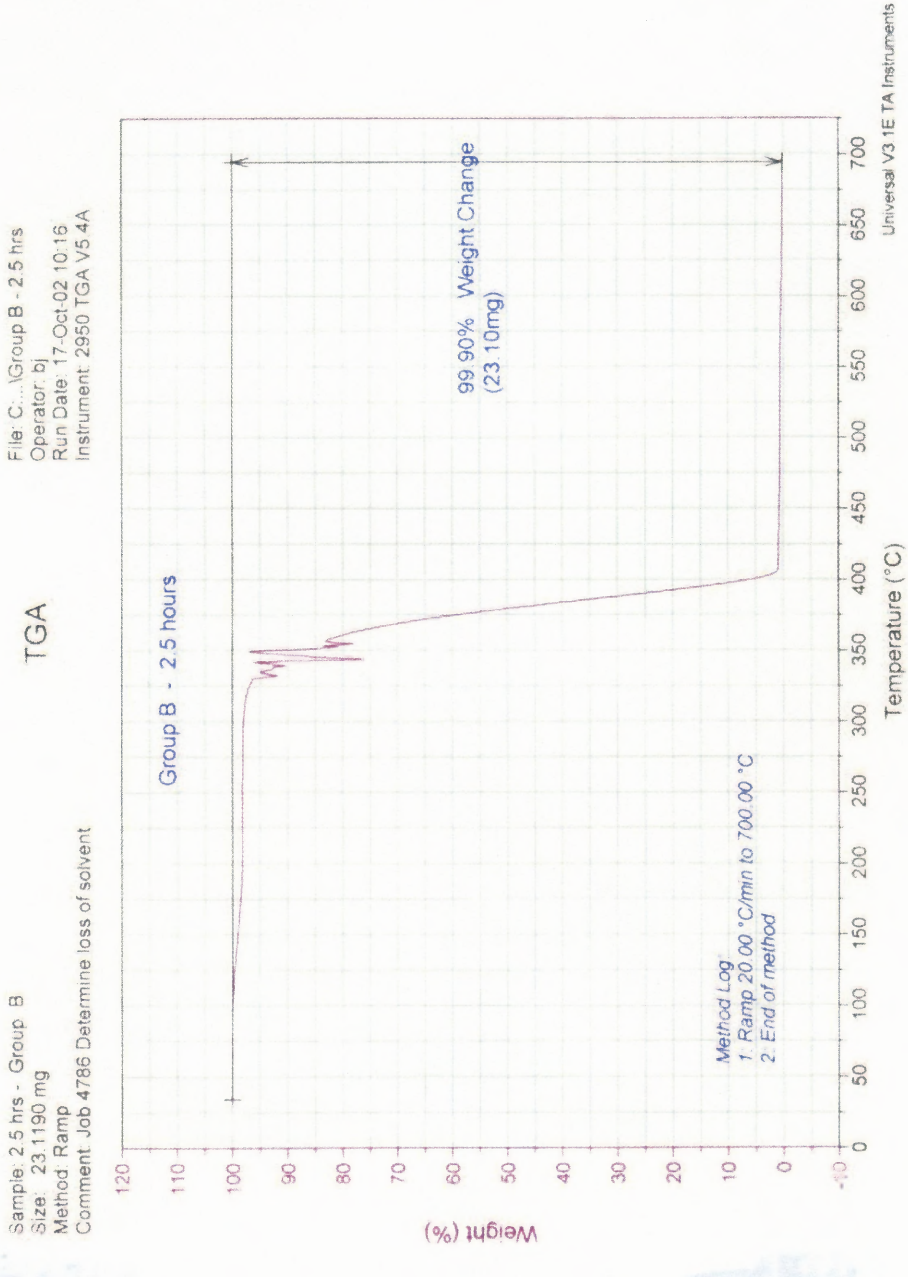

Figure B. 9 TGA testing of (Group B) 0.5 hr dried solvent cured PLA –scaffold.

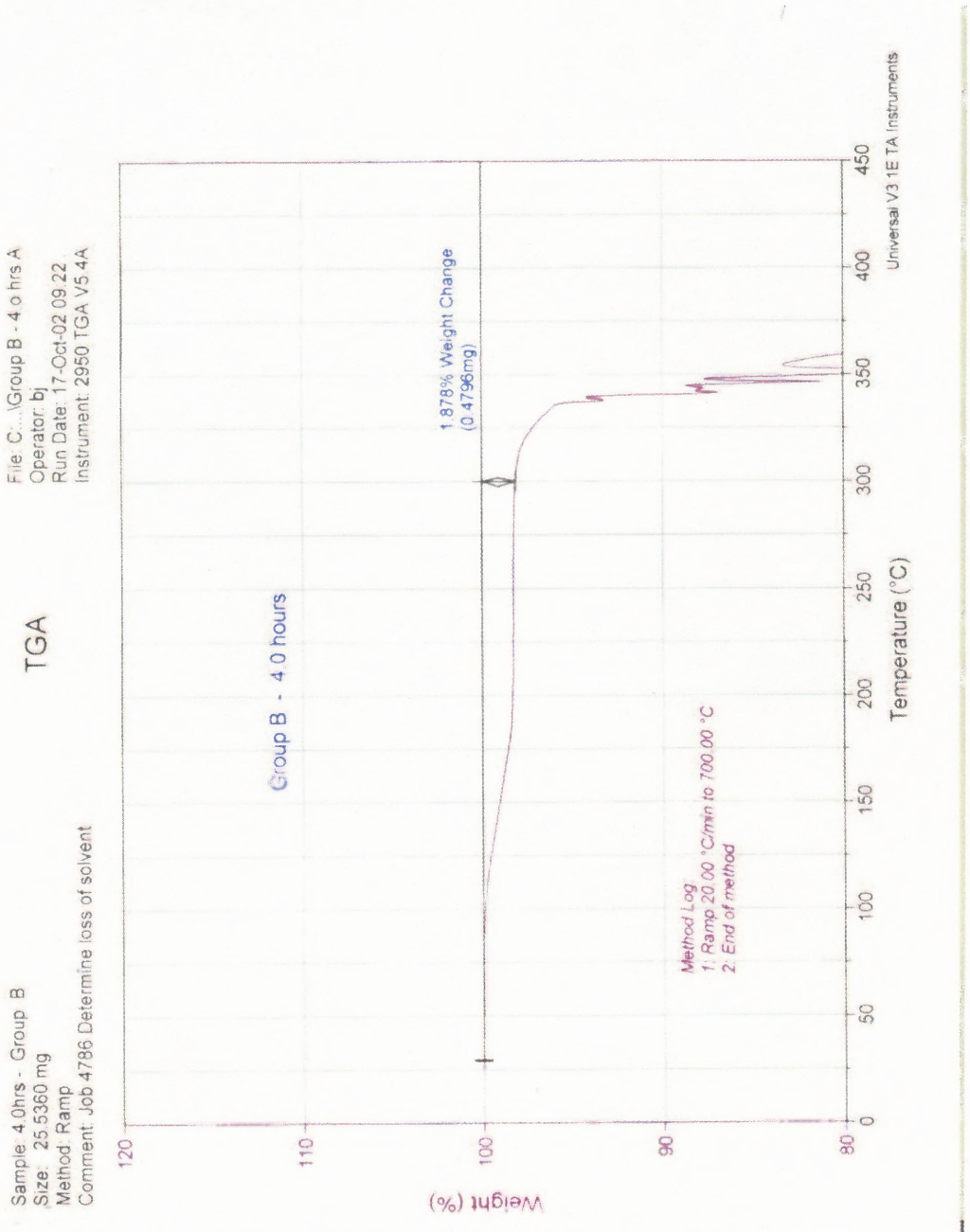

Figure B. 10 TGA testing of (Group B) 0.5 hr dried solvent cured PLA –scaffold.

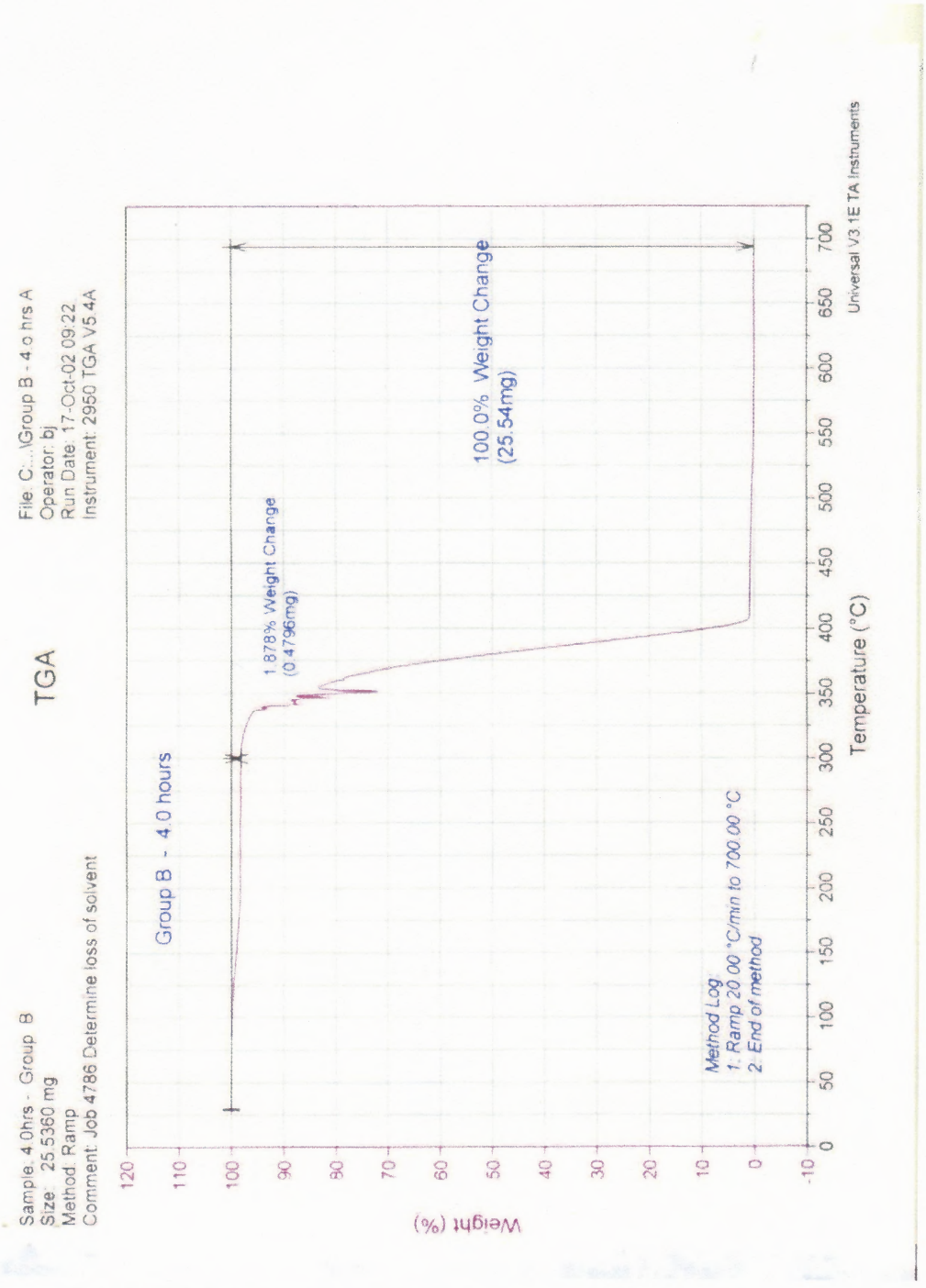

Figure B. 11 TGA testing of (Group B) 0.5 hr dried solvent cured PLA –scaffold.

APPENDIX C

DSC TEST RESULTS

DSC testing of PLA scaffold samples, this testing was done to determine if solvent is still present, and also characterize the morphology of the material.

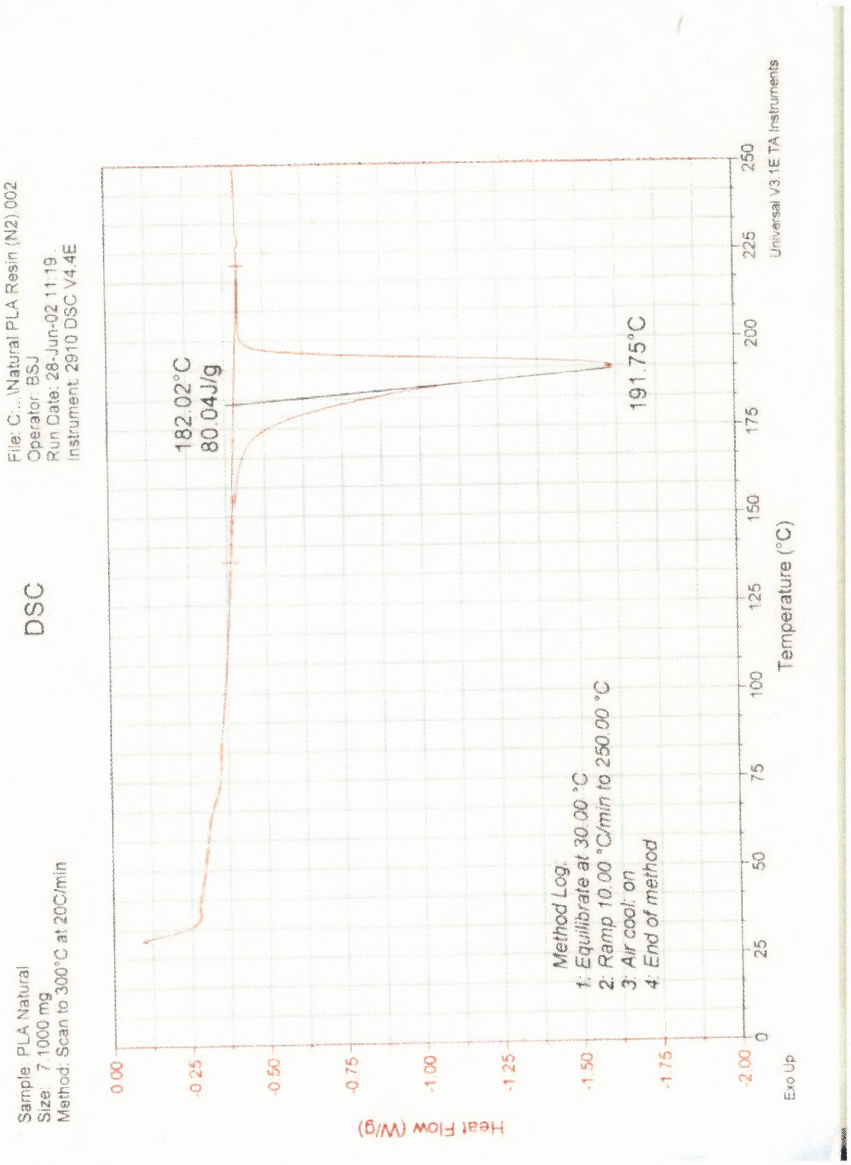

Figure C.1 DSC testing of natural PLA resin.

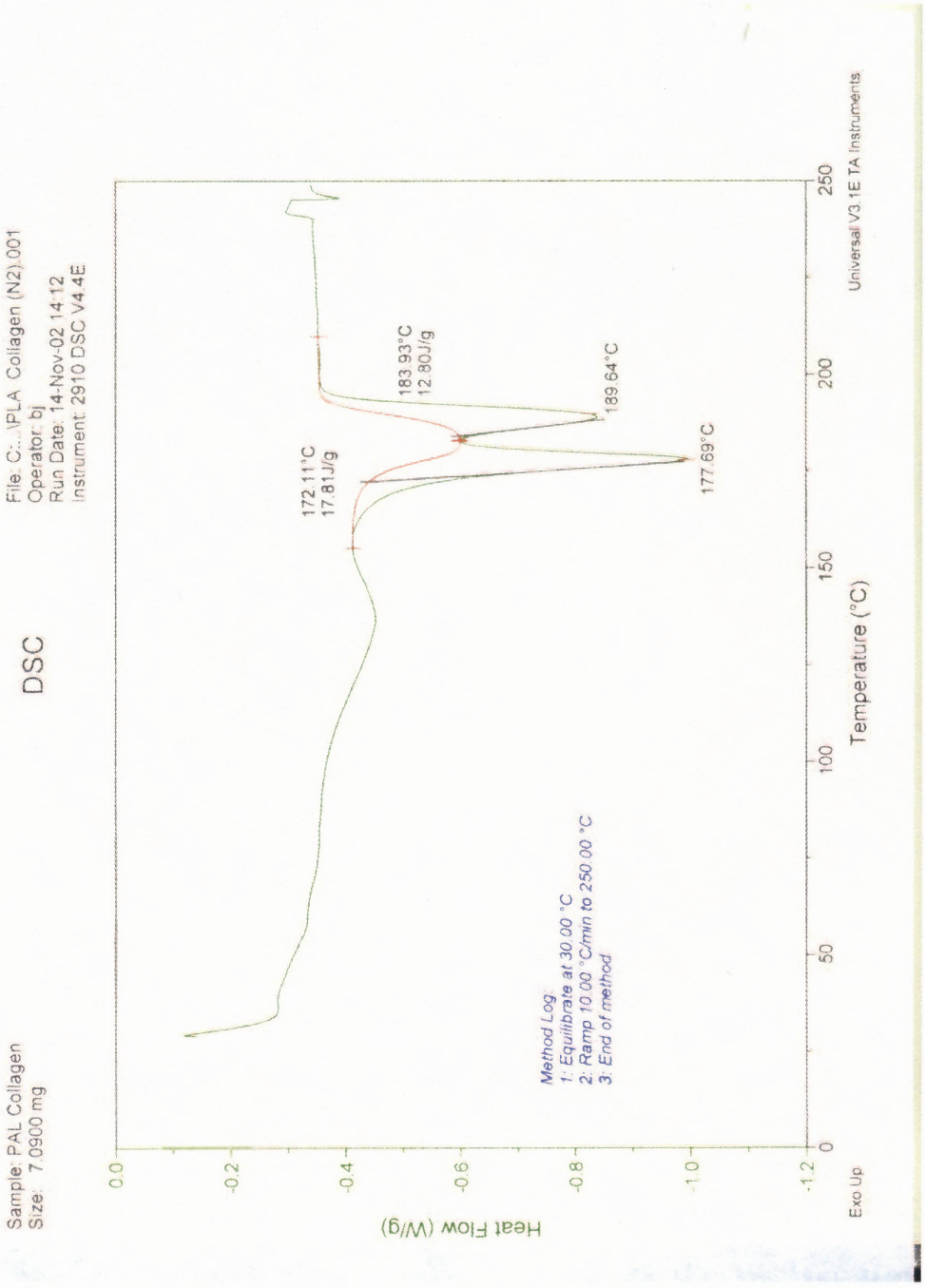

Figure C.2 DSC testing of PLA/Collagen scaffold material.

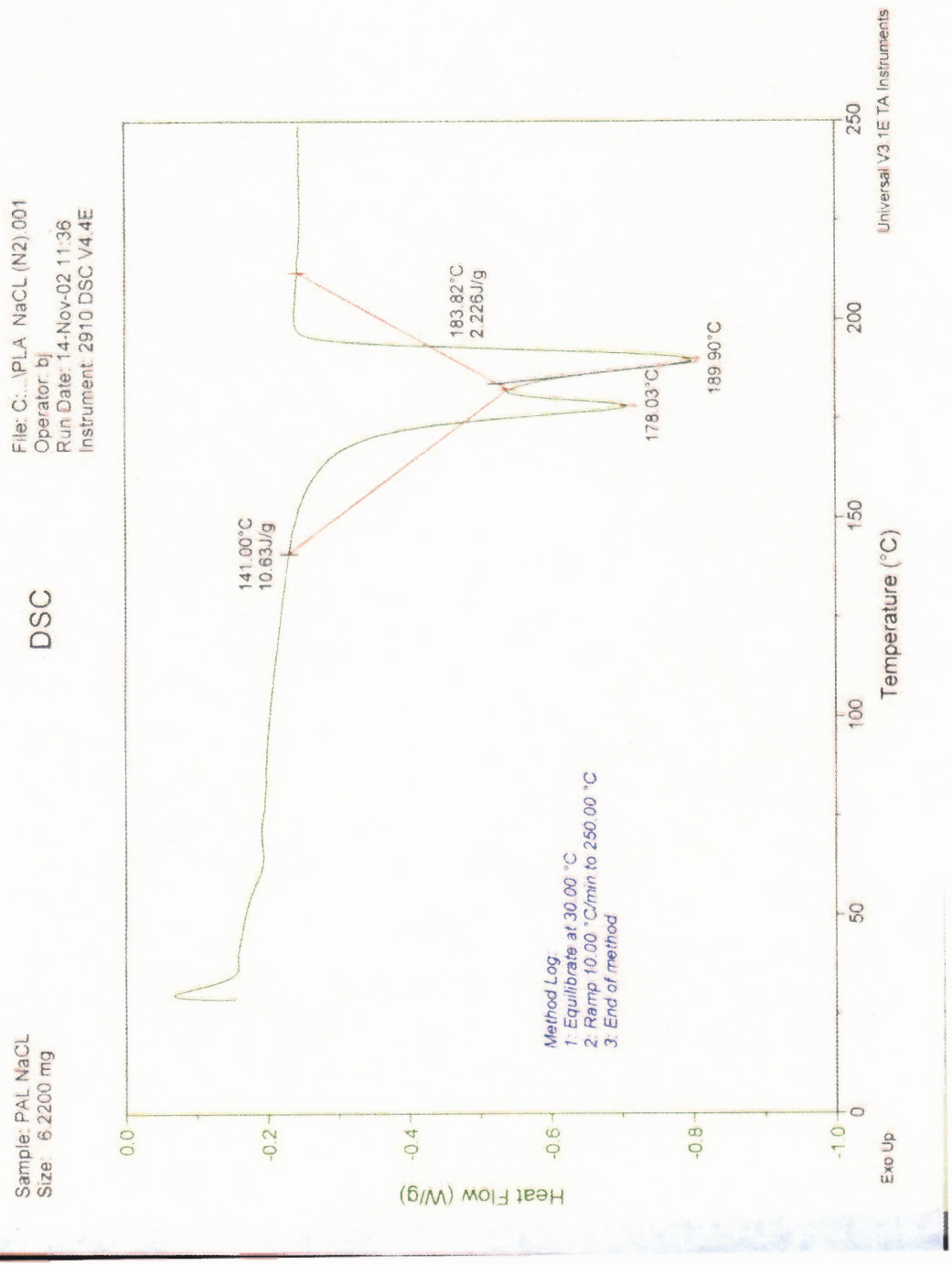

Figure C.3 DSC testing of PLA/NaCl scaffold material.

APPENDIX D

FTIR TEST RESULTS

FTIR testing of scaffold samples from all four material groups, this testing was conducted to determine the composition of each group.

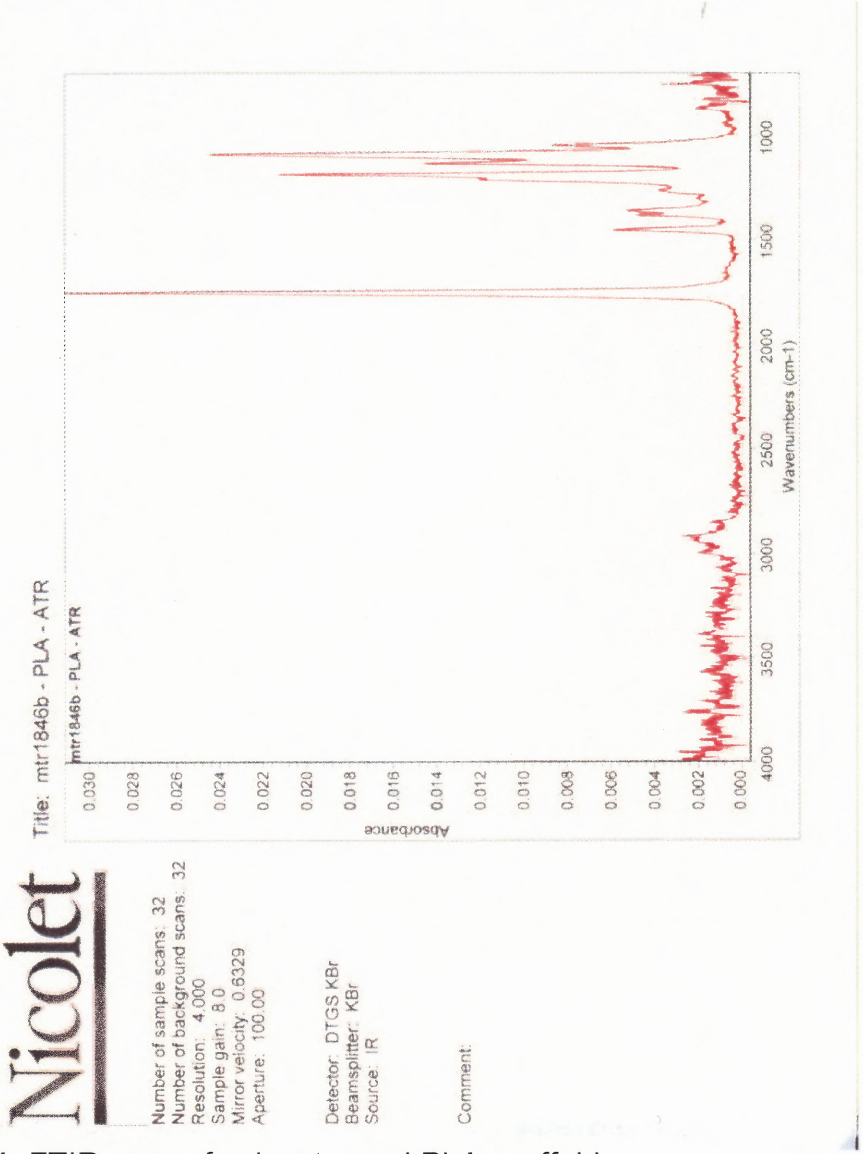

Figure D.1 FTIR scan of solvent cured PLA scaffold material.

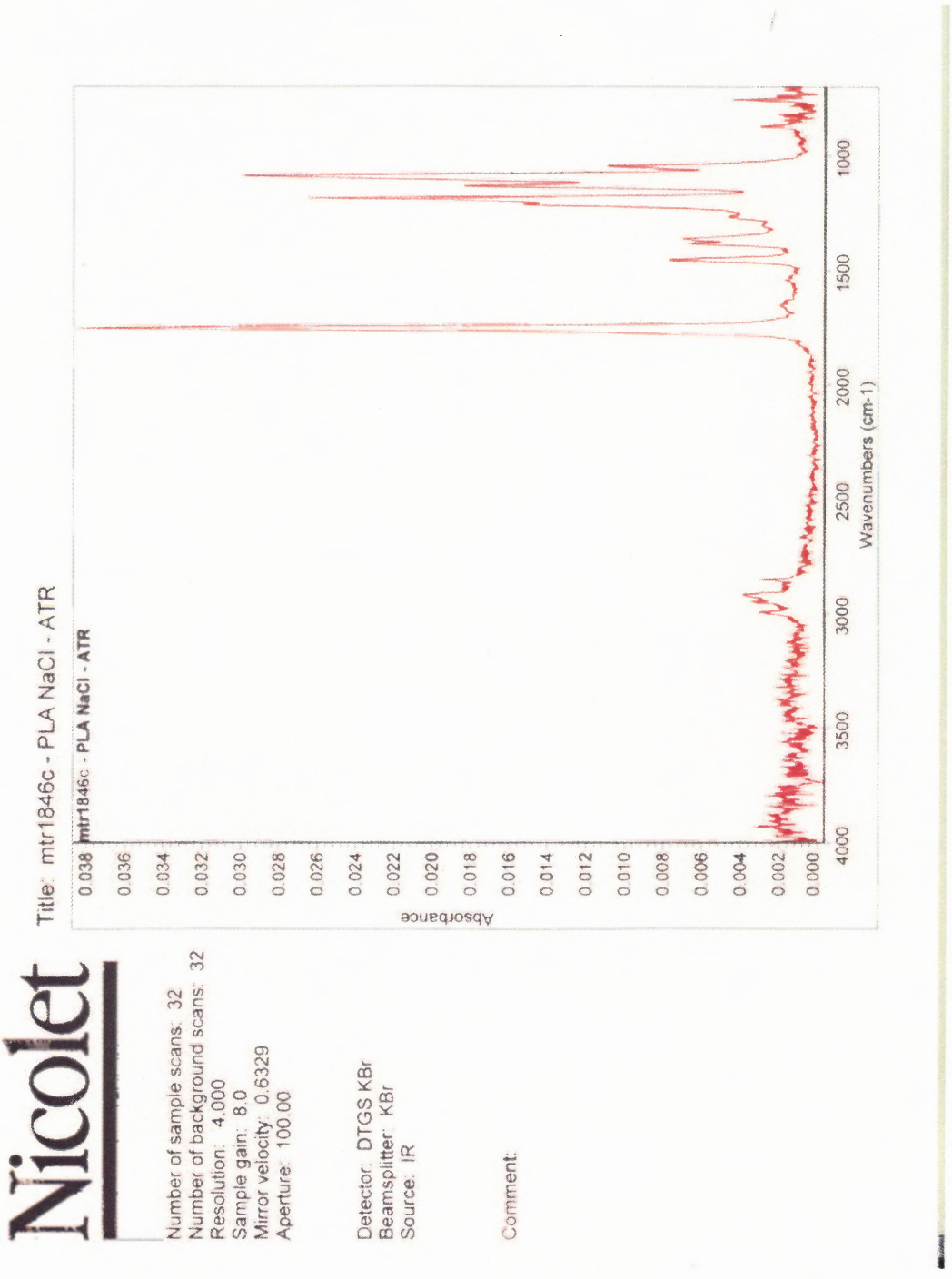

Figure D.2 FTIR scan of solvent cured PLA/NaCl scaffold material.

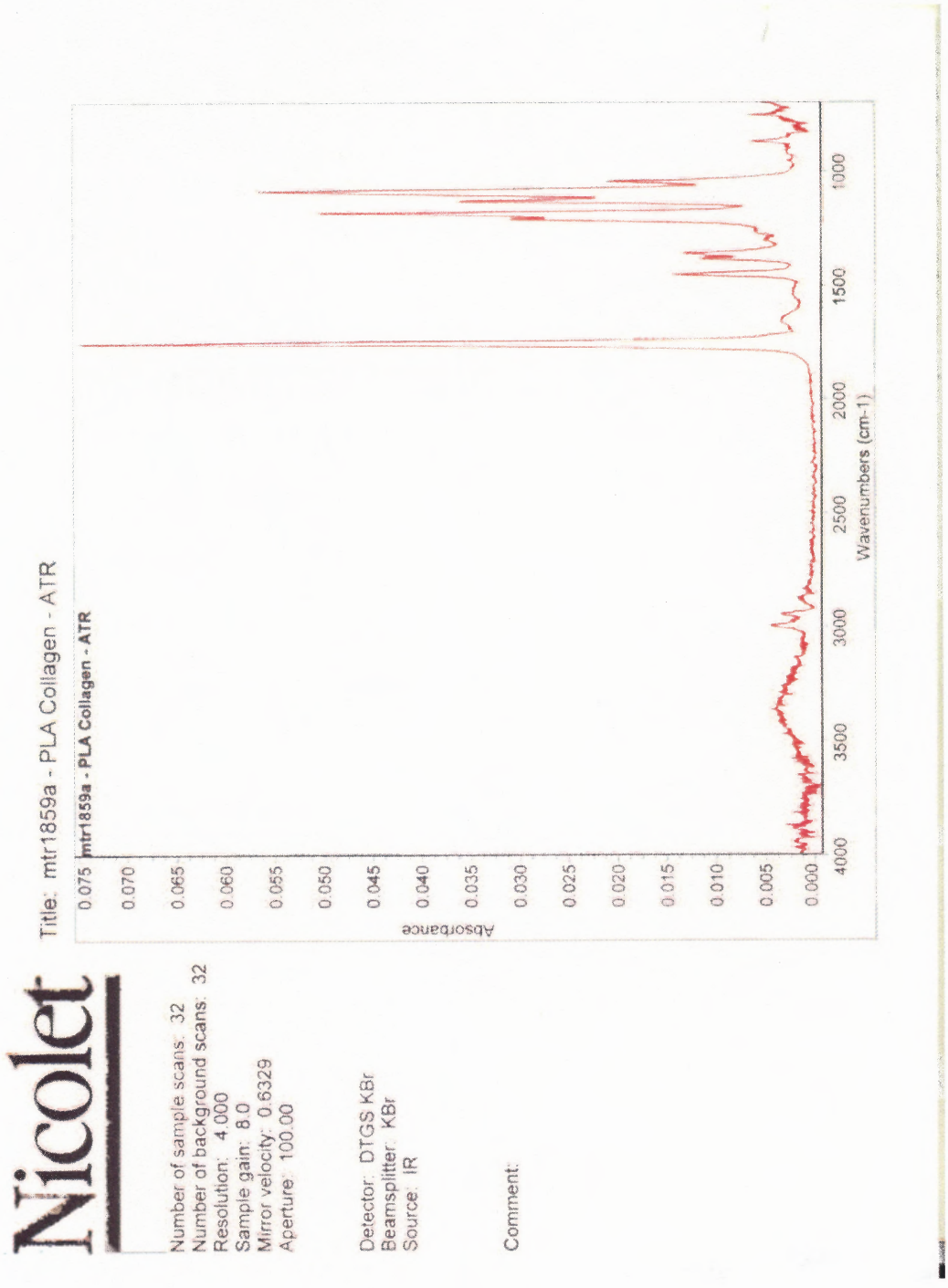

Figure D.3 FTIR scan of solvent cured PLA/Collagen scaffold material.

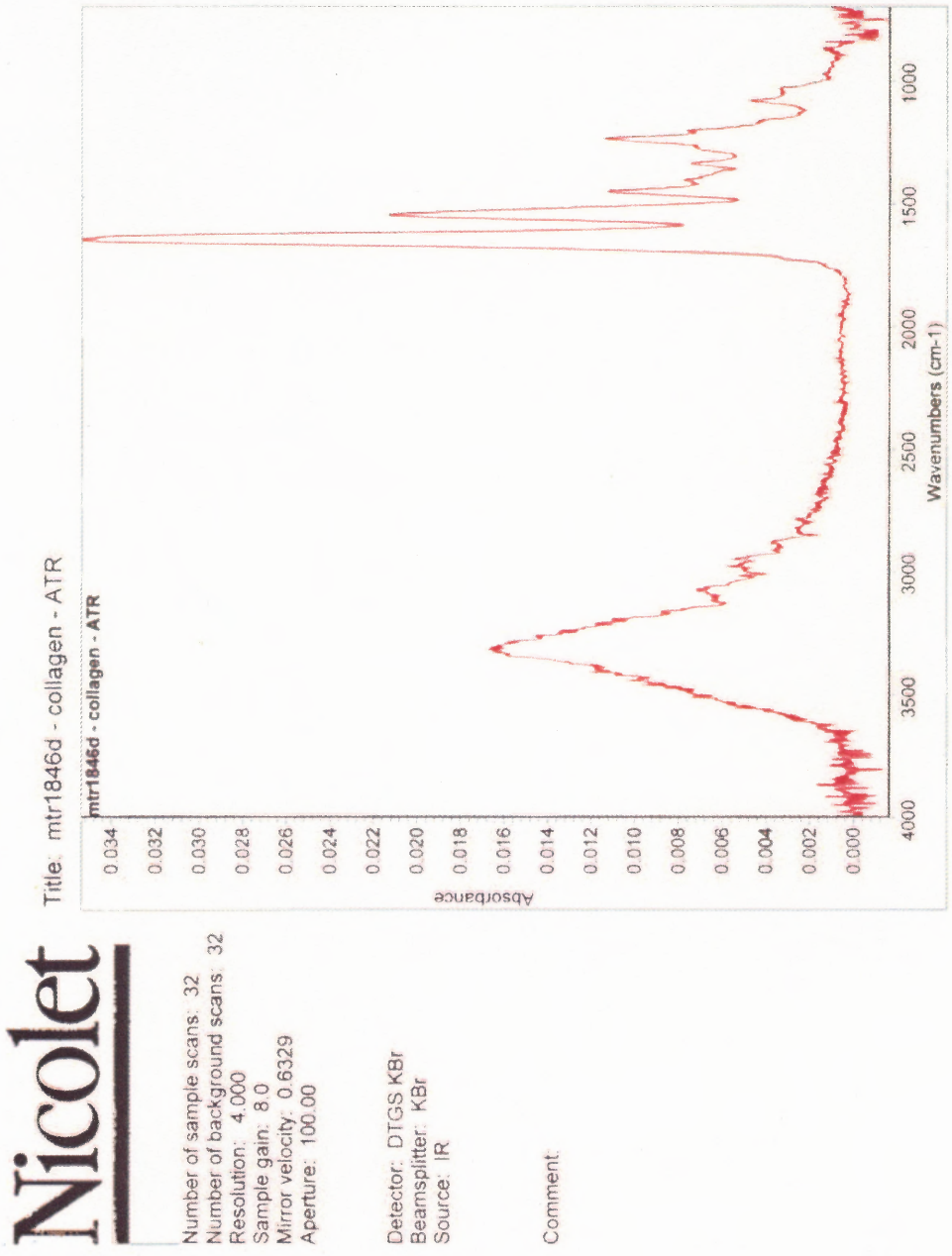

Figure D.4 FTIR scan of solvent cured Collagen scaffold material.

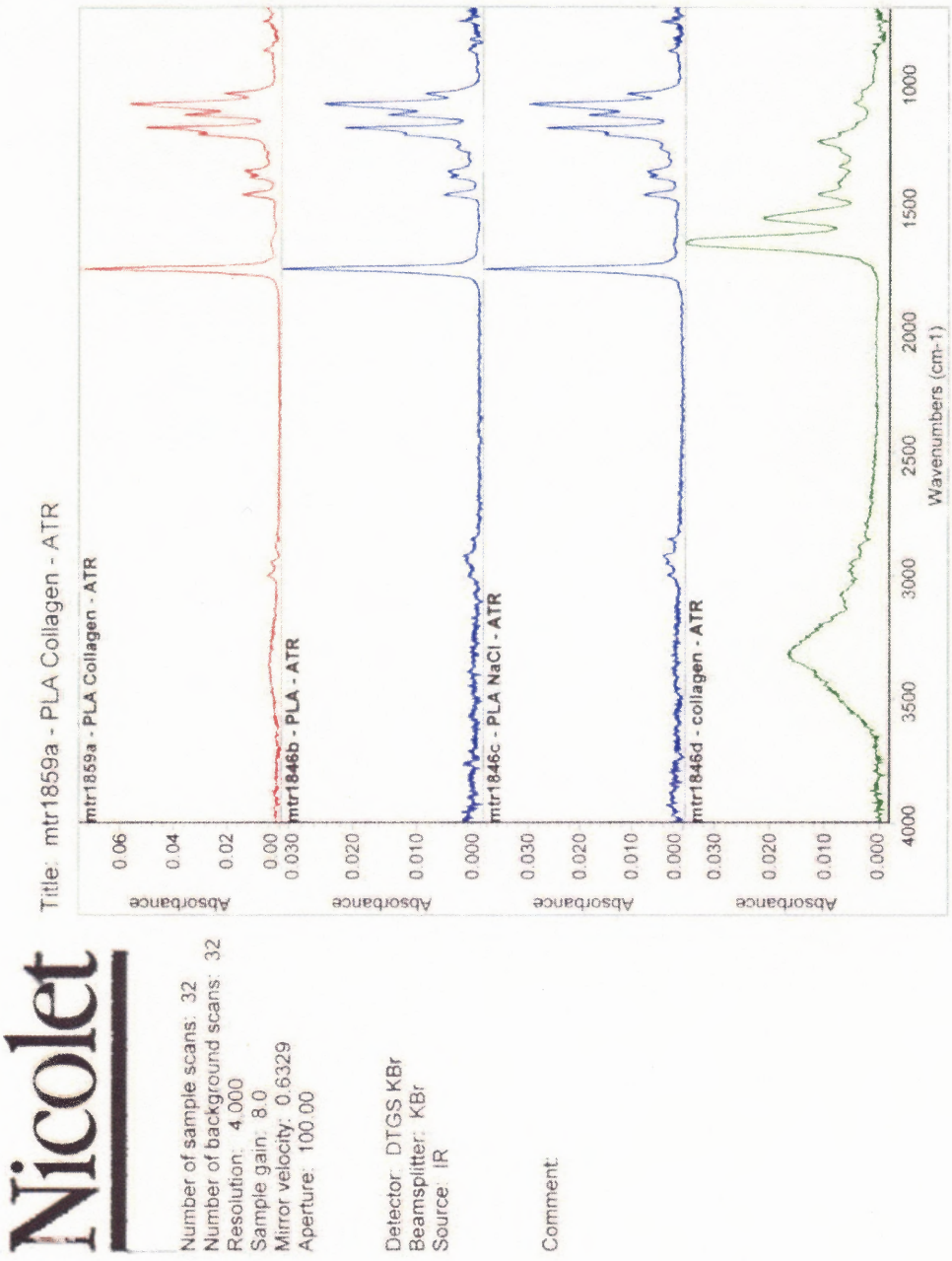

Figure D.5 Combined FTIR scans for all four scaffold materials.

APPENDIX E

SEM TEST RESULTS

SEM picture of PLA/Collagen sample, photograph was taken to visualize the variable surface of the scaffold material.

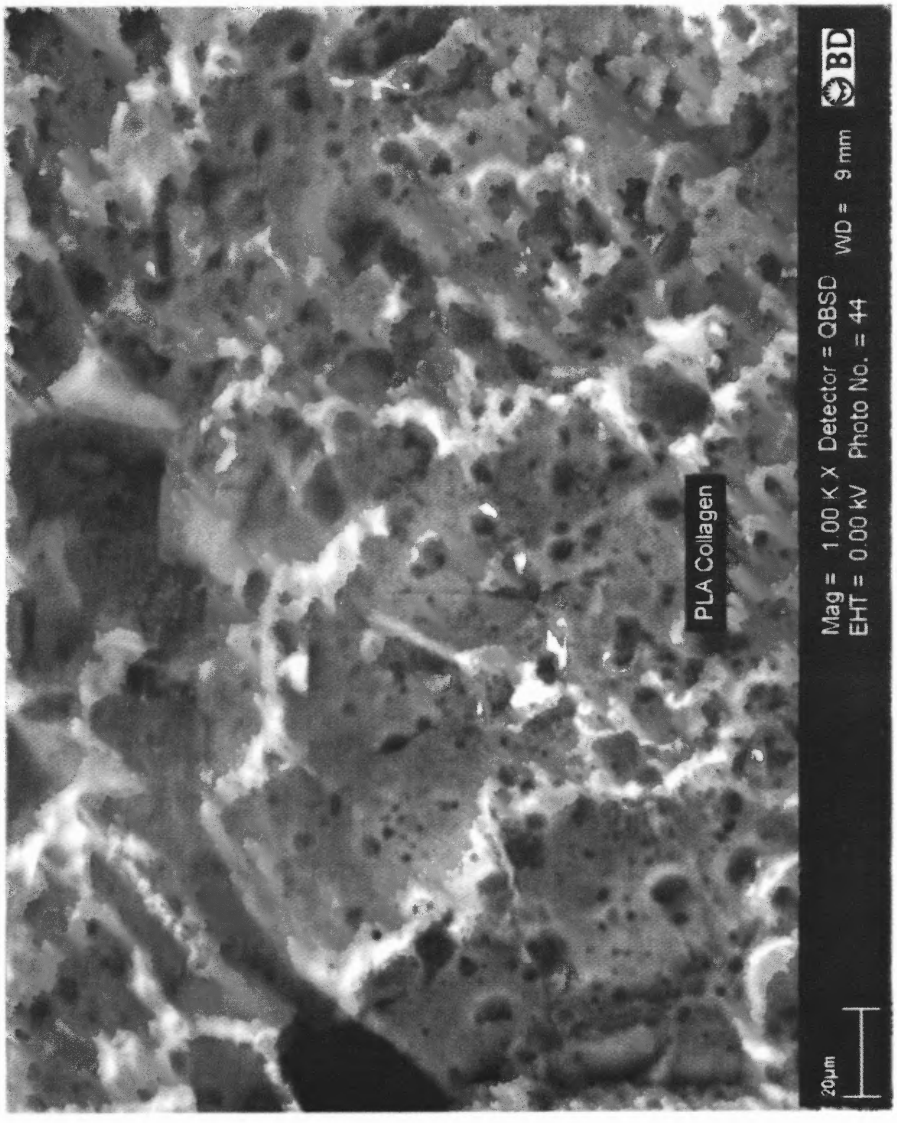

Figure E.1 SEM photograph of PLA/Collagen scaffold sample.

APPENDIX F

BIOREACTOR DESIGN

The diagram below is of the proposed heart valve bioreactor, it is designed to cycle one

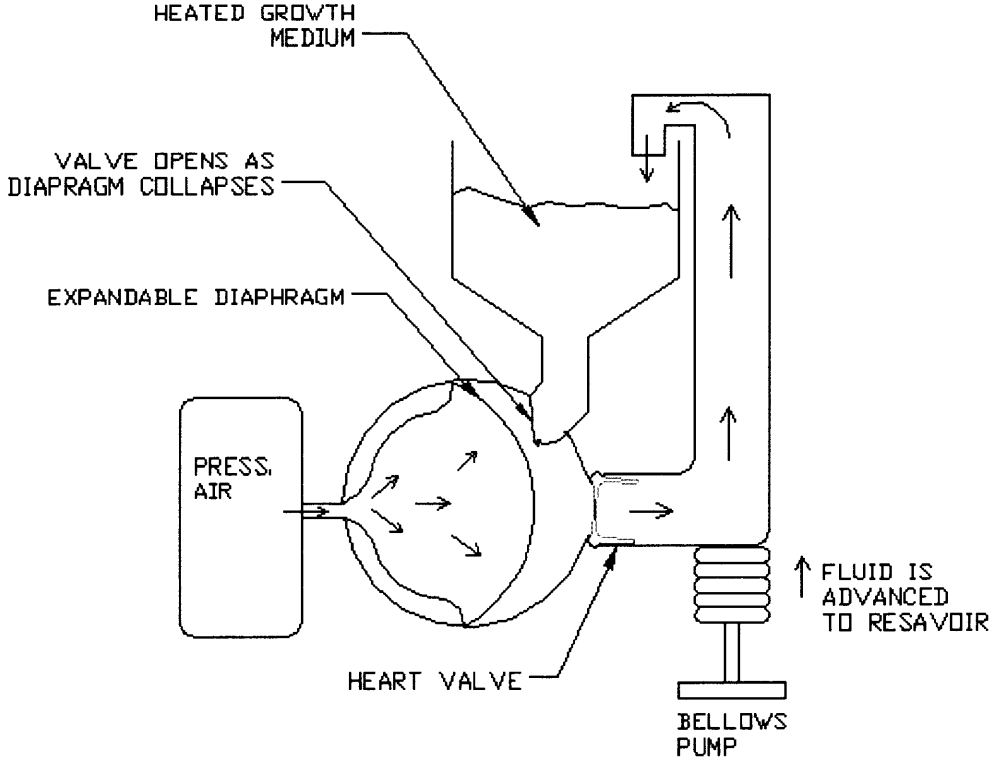

Figure F.1 Schematic diagram of proposed heart valve scaffold bioreactor.

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