

ABSTRACT

Agrawal, Pankaj Mohanlal. Design and Study of Biodegradable Small Diameter Woven Vascular Grafts. (Under the direction of Dr. Bhupender. S. Gupta)

In this research, the focus has been on designing and producing small diameter woven grafts having a biodegradable material. Woven tubes in diameters ranging from 3 to 6.5 mm are developed using a narrow width Muller loom. The material used is Polyglactin 910 biodegradable yarn of 56 denier and the structures developed are plain but with different degrees of tightness. The pick density is varied from 32 to 44 picks per inch and end density is varied by using different number of total ends.

Accordingly, the experimental work in this thesis involved weaving of 5 different sets of tubular structures and examining their behaviors. Since the woven grafts needed to be heat set to develop a circular shape that was resilient, preliminary studies were conducted on the yarns to determine heat setting conditions that led to optimum set with minimum degradation in properties. Process parameters required to manufacture such structures are identified. The effects of degradation time and structural variables on the mechanical properties of the grafts are studied. The variables were the construction parameters and the days of degradation; and the properties examined were the change in mass and thickness of the graft, and the elastic recovery, compliance and porosity properties of the structures. Statistical analysis of variance is conducted to identify significant effects in several instances.

**DESIGN AND STUDY OF BIODEGRADABLE SMALL DIAMETER
VASCULAR GRAFTS**

by

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BIOGRAPHY

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1. INTRODUCTION

A vascular disorder has been one of the most serious diseases human beings have suffered with as recorded history shows. They frequently result in either the loss of limbs or lives. The development of vascular grafts of synthetic origin has made significant progress and has allowed the reconstruction of obstructed or injured blood vessels with remarkable success. However, some serious problems still remain unsolved.^[1] While homografts are still considered to be the preferred arterial replacements, they leave much to be desired as they fall short of meeting the increasing needs of the population, due mainly to an inadequate supply, nonuniform properties, and difficulty in preparation. In long search for an ideal graft replacement, various synthetic materials have been experimented with. They included rigid nonporous tubes made of gold, silver, aluminum, glass and polyethylene.^[1] However, these materials have generally failed due to the lack of porosity and compliance. These two important requirements of vascular grafts have been partially fulfilled by incorporation of fibrous polymers and textile structures. The issues such as biocompatibility, sterilizability, handling and suturability of grafts have been satisfactorily dealt with. The needed compliance in vascular grafts has recently been achieved by the use of elastomeric fibers like spandex. In contrast to a solid tube, the inherent porous nature of textile materials provides sites for the fibrous connective tissues to grow into to achieve wall healing.

It has been shown by numerous studies that a certain amount of porosity is required in a graft in order for the latter to heal. The porosity should be such that it assists

in healing while preventing blood leakage at the same time. In order to satisfy the apparently contradictory specifications for porosity, i.e. initially low value at implantation and later high value during biological activity, concepts have been developed to use absorbable materials for vascular reconstruction. ^[2] These materials have been shown to facilitate the possibilities for regeneration of arterial tissues with minimum adverse tissue reactions. ^[3] However, the present literature lacks greatly in details that show correlation between degradation of the fabric made of biodegradable materials in physiological environment and corresponding change in the porosity and properties of the same. The objectives of this research are, therefore, to close a part of this gap. Proposed in this research are manufacture of tubular structures with a wide range of porosities; study the degradation properties of yarns; study the effect of degradation of yarns comprising the fabrics on porosity and mechanical properties; and develop predictive models for the behavior.

2. LITERATURE REVIEW

Different types of synthetic graft materials have been developed for use in small diameter vascular reconstructions when autologous vein is unavailable. Due to high surface thrombogenicity and tendency to later progressive fibrosis at the anastomotic sites the overall experience with these materials has, however, been inferior as compared to that with saphenous vein grafts. This indicates a need for coming up with alternative materials and structures in vascular prosthesis research.

Among the specifications needed for an ideal synthetic graft material the two most important parameters are proposed to be (a) low implantation porosity and (b) high healing porosity or fibroblastic permeability. In order to satisfy the apparently contradictory specifications of low initial porosity at implantation and high biological porosity during healing, Wesolowski et al.^[4] developed the concept of using absorbable materials for vascular reconstruction for the first time in 1960. In their work they used bicomponent fabrics consisting of absorbable materials. Basically there were three different designs in the Wesolowski study: collagen coated nylon or Dacron mesh, a taffeta tube with warp yarns made of Dacron and filling yarn composed of various ratios of Dacron and collagen filaments, and a taffeta tube with Dacron warp yarns and collagen core wrapped with Dacron as filling yarns. However because of either the uneven absorption of the collagen component leading to the loss of structural integrity, or the strong tissue reaction involving collagen absorption, this first generation of fabrics was not successful. To advance the composite vascular graft concept further, the

incorporation of a more biocompatible linear synthetic absorbable aliphatic polyester such as polylactide and polyglycolide as the component yarns in graft fabric were reported by Bowald et al. In their study seven to twenty cm long bypass grafts were inserted between the upper and lower parts of the descending aorta of 20 young pigs. The graft materials used were circular Polyglactin 910 mesh tubes (group I), external PTFE graft and internal Polyglactin 910 mesh tube (group II-figure 2.1.), PTFE graft (group III) and external Dacron mesh tube and internal Polyglactin 910 mesh tube (group IV-figure 2.1.). The biological activity-taking place in the grafted area was studied by light microscopy. The following are the observations made in this study: ^[5]

- The regenerative capacity of the pig aortic tissue permits 7 to 15 cm long Polyglactin 910 mesh grafts to be replaced by a new endothelialized vessel wall imitation.
- When resorbable and non-resorbable were combined to form a composite graft (groups II and IV), the result proved to be a prosthesis with an internal covering of endothelialized arterial tissue.

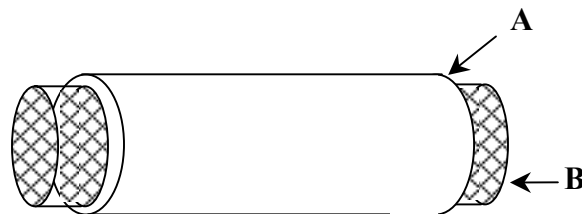


Figure 2. 1. Structure of the combined prosthesis used in groups II and IV. A, outer layer of non-resorbable material; B, inner polyglactin tube ^[5]

Greisler et al. ^[6-7] in their work on partially bioresorbable vascular grafts have compared compound prostheses with commercial grafts made of Dacron and PTFE. Conduits of 4mm inner diameter and 50 mm in length were woven from composite yarns containing 69% polyglactin910 and 31% polypropylene or 70% polydioxanone and 30% Polypropylene. Non-resorbable controls were woven Dacron and expanded polytetrafluoroethylene (ePTFE). Seventy prostheses were implanted into aortic-iliac positions of dogs, and the prosthesis/ tissue complexes were harvested serially from 2 weeks to 1 year. The following table summarizes the result obtained in this study:

Table 2. 1. Patency rates ^[6]

	2- Wk	1- Mo	2- Mo	4- Mo	6- Mo	12- Mo	Patency Rate (%)
PG910/Polypropylene	1/2	2/2	3/3	1/1	2/2	9/10	90
Polydioxanone/Polypropylene	2/2	1/1	2/3	2/2	1/2	11/12	86
Dacron	2/2	0/1	2/3	1/1	1/2	7/10	68
ePTFE	2/2	1/1	2/3	1/1	0/2		54

It can be seen from the results that resorbable grafts as a group had significantly greater patency than control grafts.

Uretzky et al. ^[8] developed a compound vascular prosthesis in which knitted Dacron graft was coated with a polymeric biodegradable sealant. Polyethylene oxide-poly-lactic acid segmented copolymers comprised the degradable component of the graft. The prostheses were completely watertight, and significant degradation started after 1 week, with absorption completed in 3 weeks. Seven woven and six knitted polyethylene oxide-poly-lactic acid-coated Dacron grafts as extracardiac conduits (16 mm), connecting

right ventricle and the pulmonary artery were implanted in dogs. The biodegradable polymer was fully degraded and exhibited a complete incorporation of the compound vascular prosthesis. The results of this study are given in table 2.2. This study indicates the superior healing properties of the selectively biodegradable grafts, which might increase long-term patency and decrease complication of right ventricular conduits.

Table 2. 2. Findings of Right Ventricle-Pulmonary Artery Conduits^[8]

Group	No.	Period	Thrombus	Peel Detachment	Incomplete Surface
A. Woven Dacron	7	12-18 mo, m= 4	3/7	5/7	4/7
B. Coated Knitted	6	12-18 mo, m=13	0/6	0/6	0/6

2.1. Porosity and vascular Healing

Autogenous vessels or collagen tubes remain patent in solid form but porosity is an essential component for function of small diameter synthetic vascular grafts. This observation has been uniformly confirmed and to date no solid wall synthetic graft has been fabricated that will function successfully as a small diameter graft. This is because porosity is an essential requirement for the transfer of fluid and ions through the graft wall thickness and provide as a scaffold for ingrowth of perivascular tissues.

The influence of porosity in vascular grafts healing was first recognized by wesolowski et al.^[2] in 1961. Based on a 3 year study involving over 350 growing pigs

and dogs, selected human subjects, and 37 different synthetic fabrics, they proposed that highly porous grafts would have complete and rapid neointima formation without subsequent degenerative changes. In 1972, expanded polytetrafluoroethylene (ePTFE) grafts were introduced. Matsumoto et al.^[9] reported improved patency rates using ePTFE as an arterial substitute in a canine model. This study concluded that the marked improved patency was based on the derived antithrombogenicity of the cellular neointima and that the high porosity was responsible for neointima formation.

In 1986, Clowes et al.^[10] reported the results of a primate study involving 4-mm PTFE grafts, 6-8 cm in length, and introduced the concept of transmural capillary ingrowth demonstrated by figure 2.2. In their study, the endothelial coverage of graft surface was radically altered simply by changing the porosity of the graft from 30 μm (standard) to 60 μm (internodal distance). In the low porosity grafts, the endothelium was derived from adjacent artery and grew as a continuous monolayer toward the center of the graft for approximately 1 cm. The central zone of these grafts remained unhealed and was covered with small clumps of thrombus and leukocytes, so-called pseudointima. In the high porosity grafts, capillaries were derived from surrounding granulation tissue, which penetrated the graft matrix (transmural) and provided multiple sources of endothelium at the luminal surface.

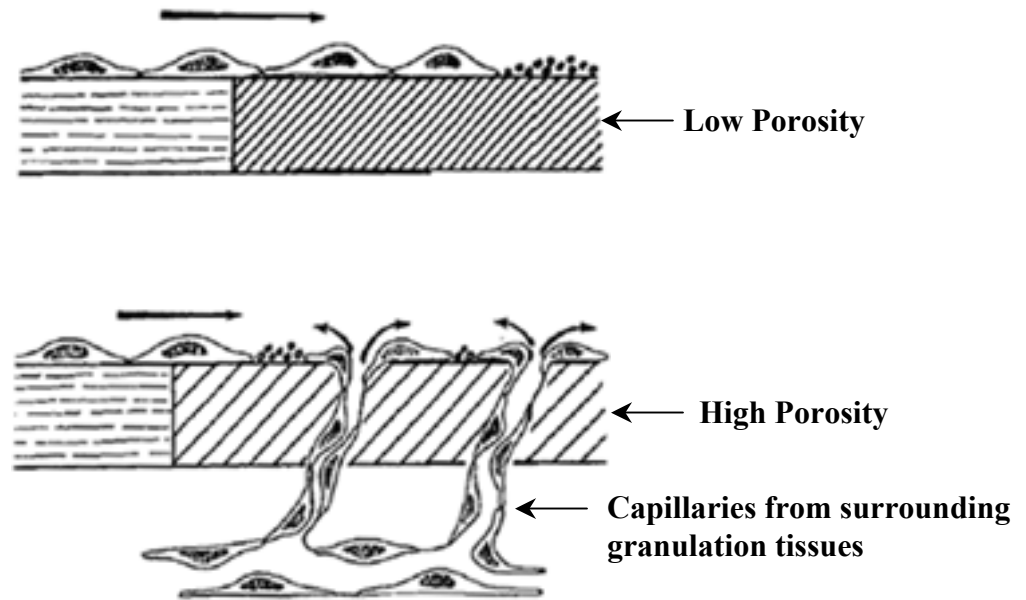


Figure 2. 2. Diagrammatic representation of healing by endothelium (and SMCs) of low porosity (top) and high porosity (bottom) PTFE grafts. In low porosity grafts endothelium is derived from the cut edges of adjacent artery and grows as a continuous monolayer toward the center of the graft . In high porosity grafts, capillaries derived from surrounding granulation tissue penetrate the graft matrix and provide multiple sources of endothelium at the luminal surface.¹⁰

Significant role of porosity of grafts in neoarterial wall regeneration is discussed in a study by Kiyoshi et al.^[11] involving segmented polyurethane grafts with or without micropores. Three different models of grafts, namely model I (microporous, permeable, compliant), model II (smooth surfaced, impermeable, compliant), and model III (smooth surfaced, nonpermeable, noncompliant), were implanted into aortas of rats for upto 24 weeks, and the neoarterial regeneration process was studied. Table 2.3. summarizes the patency rates of the three graft models during the entire course of implantation.

Table 2. 3. Patency Rate^[11]

	4 weeks	12 weeks	24 weeks	Patent (Nonstenotic)	Patent (Stenotic)	Occluded	Patency Rate
Model I	9/9 (0)	4/4 (0)	5/5 0	18/18 100%	0/18 0%	0/18 0%	18/18 100%
Model II	5/7 (0)	4/4 (2)	5/5 (2)	10/16 63%	4/16 25%	2/16 12%	14/16 88%
Model III	6/7 (0)	2/5 (2)	2/5 (2)	6/17 35%	4/17 24%	7/17 41%	10/17 59%

(): The number of grafts with macroscopically significant stenosis (narrowing or constriction of the graft)

One hundred percent patency rate for all implantations periods was achieved for model I grafts, whereas a remarkably low patency rate was observed for model III grafts. Model I grafts were free from macroscopic stenosis whereas the lowest nonstenotic patency rate was found for model III grafts. These results suggest that porosity cooperated synergistically to enhance neoarterial wall regeneration.

Porosity refers to the proportion of void spaces (pores) within the boundaries of a solid material, compared to its total volume and is given by the following equation (1):

$$P = 100 \left[1 - \frac{M}{L\rho_m} \right] \quad (1)$$

Where, P = Porosity (%)

ρ_m = Density of solid material (g/cm³)

M = Mass per unit area of porous material (g/cm²)

L = Thickness of porous material (cm)

Wesolowski ^[2] assessed the porosity of fabric vascular prostheses by measuring the flux of water through the wall of the material at a pressure differential of 120 mmHg (ml/cm² min). Porosity in vascular prosthesis is, however not constant. These structures are not designed to be rigid structures. They are compliant and compressible for ease of handling and suturing, and can be readily deformed by radial and longitudinal tensions as well as by internal pressure. Such stresses not only change the dimensions of the prosthesis but also redistribute the yarns and fibers within the fabric. This affects the distribution, size, and tortuosity of the channels that carry fluid from one side of the prosthesis wall to the other. Consequently, when exposed to the hydrostatic pressure gradient of the water permeability test, most prostheses experience some loss in thickness and porosity over time, due to compaction of the structure. The best description of the porous nature of a vascular substitute includes permeability, pore size, dimensions of the interconnections between pores and the pore to solid volume ratio of the graft structure, so that studies can be compared and interpreted correctly.

In a study titled “Theoretical determination of porosity in fabrics and its application to the design of woven vascular grafts”, Pourdeyhimi et al. ^[12] have suggested the following equations for estimating the porosity of a material.

$$P_1 = \frac{1}{n_1} - \frac{0.0041020N_1}{\rho_1} \quad (2)$$

and,

$$P_2 = \frac{1}{n_2} - \frac{0.0041020N_2}{\rho_2} \quad (3)$$

Where, P_1, P_2 = Pore size in the warp and weft directions respectively (m)

n_1, n_2 = Warp and weft threads per cm

N_1, N_2 = Linear density of warp and weft yarns respectively (Tex)

ρ_1, ρ_2 = Fiber density of warp and weft threads respectively (g/cm³)

Based on these equations, the porosity of woven graft can be designed to vary from zero to any desired size. Such a theoretical calculation could act as a tool to control porosity of woven vascular grafts. Table 2.4. illustrates the maximum weavable constructions that have been obtained from the above given equations.

Table 2. 4. Pore Sizes I Maximum Weavable Constructions When N= 18^[12]

n_1	T_1	n_2	T_2	P_1	P_2	P_1P_2	P_d	%p
67	0.0149	34	0.0294	0.375	145.239	54.46	8.3	0.12
66	0.0151	34	0.0294	2.636	145.239	382.85	22.1	0.86
64	0.0156	34	0.0294	7.371	145.239	1070.56	36.9	2.33
*60	0.0167	34	0.0294	17.788	145.239	2583.51	57.3	5.27
59	0.0169	34	0.0294	20.613	145.239	2993.81	61.7	6.00
**56	0.0178	34	0.0294	29.692	145.239	4312.5	83.6	8.21
54	0.0185	34	0.0294	36.306	145.239	5273.05	81.9	9.68
52	0.0192	34	0.0286	43.429	136.835	5942.61	86.9	10.82
49	0.0204	35	0.0286	55.203	136.835	7553.7	98.1	12.95
48	0.0208	35	0.0286	59.454	136.835	8135.54	101.7	13.67
45	0.0222	35	0.0278	73.343	128.899	9453.84	109.7	15.31
42	0.0238	36	0.0278	89.216	128.899	11499.85	121.0	17.39
40	0.0250	36	0.0263	101.121	114.279	11556.01	121.3	17.56
38	0.0263	38	0.0250	114.279	101.121	11556.01	121.3	17.56
35	0.0286	40	0.0217	136.835	68.512	9374.84	109.2	15.09

N=Tex value of warp and weft threads

n_1 = warp threads per cm

T_1 =thread spacing in the warp direction (cm)

n_2 = Weft threads per cm

T_2 =thread spacing in the weft direction (cm)

P_1, P_2 = Pore size in the warp and weft direction respectively (μ m)

P_d = Equivalent pore diameter (μ m)

%p= Percentage Porosity

* This corresponds to Woven Cooley Verisoft[®] vascular graft manufactured by Meadox Medicals

** This corresponds to Woven DeBakey[®] vascular grafts manufactured by USCI

However, fewer attempts been made to examine porosity and model changes in it as yarn degrades and fabric changes composition. It would, therefore, be useful to generate such data and have a model that could predict pore size and its distribution from non-contact measurements on a graft specimen.

2.2. Polymeric biomaterials for Vaculatures

2.2.1. Selection

Polymers are a promising class of biomaterials that can be engineered to meet specific end use requirements. They can be selected according to key characteristics such as mechanical strength, flexibility and elasticity, degradability, permeability, solubility and transparency.

Any fibrous structure that is intended to be used for a vascular prosthesis should consist of the following properties^[13, 14, 15]

1. Be non-thrombogenic
2. Allow little or no blood loss (homeostatic)
3. Be biocompatible or hemocompatible
 - Be non-destructive to blood cells and enzymes
 - Should not cause depletion of the blood electrolytes
 - Have no adverse immune responses
 - Should not alter plasma proteins
 - Be non-cytotoxic
4. Be sterilizable without adverse affect on properties

5. Have low rate of infection when exposed to bacteria
6. Have desirable range of tensile strength
7. Have an optimum level of resilience, fatigue endurance and biostability. This means it should retain its chemical and mechanical properties such as compliance and elasticity during functional use
8. Should possess a certain minimum level of porosity

From the point of view of biostability or bio-absorbability, polymeric biomaterials can be divided into two groups

- Biodegradable polymers
- Non-biodegradable polymers

A variety of terms describing biodegradation of polymers are used, including degradation, bioabsorption, bioerosion and resorption.^[16] The term biodegradation can be used in a wide sense for any degradation occurring in a biological environment. A bioinert material does not elicit severe foreign body reaction, such as activation of the immune system or blood coagulation leading to thrombus formation.

Microscopic degradation of polymeric materials can take place either by random chain scission (which remarkably reduces the MW and mechanical strength with an insignificant loss of mass) or by zipper like scission from polymer chain end, in which case mass and the molecular weights decrease linearly. If a detectable loss of mass takes place, even if it occurs at a much later stage than the stage during which molecular weight reduction takes place, then the polymer can be regarded as bioresorbable. The

latter is a polymer that degrades into water soluble, low molecular weight compounds such as monomer units, which are then absorbed by surrounding body fluid. If these compounds interact with cells surface or penetrate cell membranes, such an absorbable polymer might give an adverse reaction. Therefore the selection of a bioabsorbable polymer must be made with much more caution than needed for selection of nonbioabsorbables.^[16]

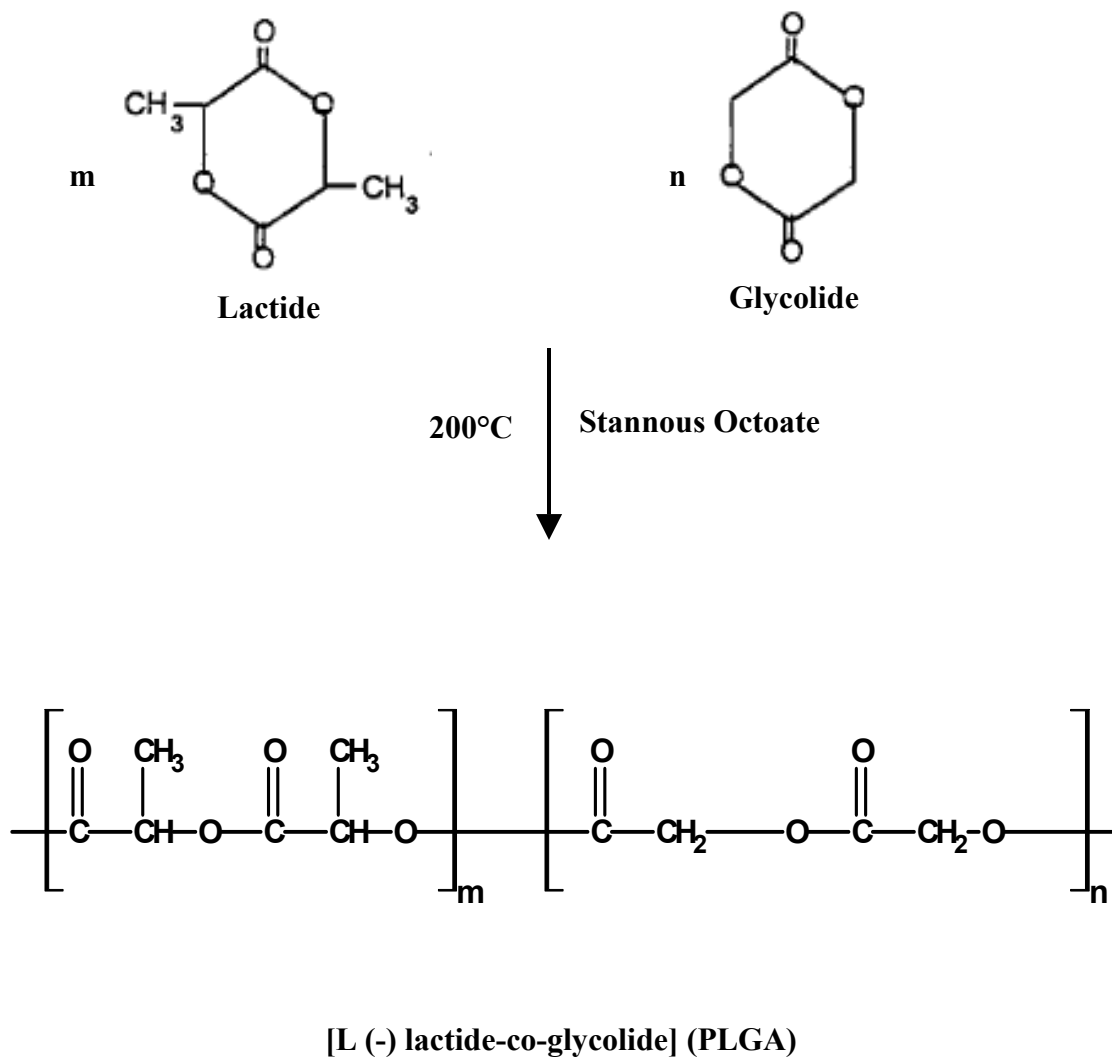
2.3. Poly [L (-) lactide-co-glycolide] (PLGA)

2.3.1. Synthesis

Poly [L (-) lactide-co-glycolide] compositions suitable for the manufacture of absorbable suture and yarn are copolymers prepared by heating in the presence of stannous octoate catalyst at about 200°C at least 65 mol percent L (-) lactide and no more than 35 mol percent of glycolide. Absorbable sutures prepared from copolymers of glycolide and L (-) lactide using the catalyst stannous octoate have amount of L(-) lactide in the range of 10 to 15 mol percent and glycolide in the range of 85 to 90 mol percent.

Both the L (-) lactide and glycolide used for the polymerization reaction should be pure and dry. The reaction is run in dry container under a blanket of dry nitrogen. Stannous octoate is used to catalyze the polymerization. The ratio of monomer to catalyst (mol/mol) is desirably in the range of about 50,000-150,000:1. A minor quantity of glycolic acid may be present as a chain-stopping reagent to control the molecular weight. If glycolic acid is used the ratio of monomer to glycolic acid may be in the range of 400:1

to 2,000:1. To effect polymerization, the mixture of L (-) lactide and glycolide is heated at a temperature of about 200°C for about 5 hours until a sample of the polymer has an inherent viscosity of about 1.4 at 0.1 percent concentration in hexafluoroisopropanol at 25°C. ^[17]



2.3.2. Properties

The Physical and chemical properties of PLGA with 90 % glycolide and 10% L (-) lactide composition are presented in table 2.5.

Table 2. 5. Typical Chemical and Physical Properties of Poly [L (-) lactide-co- glycolide] (PLGA)^[18,19,20]

Property	Units	Value
Polymer type	-	Saturated polyester
Morphology	-	Orthorhombic (a 5.25Å, b 6.24Å, c 7.02Å)
Molecular formula	-	$[(C_3H_4O_2). (C_2H_2O_2)]_n$
Density	g cm ⁻³	1.548
Melting Temperature	°C	210-217
Glass Transition temperature	°C	37
Dry tenacity	g/denier	6.55
Breaking Elongation	(%)	18.44
Specific work of rupture	N/Tex	5.46×10^{-2}
Modulus of Elasticity	g/denier	67.5

2.4. Degradation Behavior of PLGA

Degradation of synthetic polymers inside the human body or in simulated biological environment can be intentional and unintentional. In the former, polymers are intentionally designed to degrade within the body for the purpose of either minimizing adverse reactions, such as foreign body reaction that the polymer could cause or serving the designated function through degradation. The polymers used to close wound and to deliver drugs are two typical examples. Other applications could be to use as temporary scaffolding material in tissue engineering.

In case of PLGA copolymer, it contains hydrolytically unstable, linear aliphatic ester bonds, which facilitate hydrolytic degradation by body fluids. The degradation mechanism of this class of polymers proceeds through two main stages: first in the amorphous region and then in the crystalline regions. Hydrolytic degradation starts in amorphous regions, as the tie chain segments in these regions degrade into fragments. Along the scission of the tie-chain segments which connect the crystal blocks in the axis (fiber) direction a lower tensile strength is observed.^[21]

2.4.1 In *Vitro* Degradation of PLGA

The Systematic development of *in vitro* models of the physiological environment is of the utmost importance in understanding the basic mechanism *in vivo*. In vitro modeling allows the physiological environment to be simplified such that effects of specific parameters such as pH, temperature and enzyme can be evaluated.

2.4.1.1. Effect of pH on Hydrolytic Degradation of PLGA

As discussed earlier the presence of aliphatic ester bonds in PLGA materials renders them as hydrolytically unstable units. They degrade by hydrolysis from body fluids.

Extensive research has been done in past to evaluate how this material degrades in a physiological saline solution at different pH levels. A study done by Chu et al.^[22] on PLGA suture shows that PLGA copolymer exhibits unique hydrolytic degradation at various levels of pH. It degrades significantly faster in a highly alkaline buffer than in acidic and physiological buffers as can be seen from the results in table 2.6.

Table 2. 6. Tensile Properties of 2-0 Vicryl Suture Material at Different Levels of pH ^[22]

Days	Breaking Strength (gpd)			Breaking Elongation (%)			Specific Work of Rupture (J/g)		
	pH			pH			pH		
	5.25	7.44	10.09	5.25	7.44	10.09	5.25	7.44	10.09
0	6.27	6.27	6.27	15.88	15.88	15.88	47.65	47.65	47.65
	±0.18	±0.18	±0.18	±0.80	±0.80	±0.80	±0.16	±0.16	±0.16
7	5.42	5.74	4.00	15.012	15.12	14.44	33.97	37.5	15.93
	±0.29	±0.30	±0.58	±0.99	±1.23	±0.97	±0.11	±0.23	±0.18
14	4.01	5.12	1.74	12.03	13.84	13.01	18.25	29.73	8.45
	±0.59	±0.35	±0.13	±1.09	±0.94	±0.82	±0.20	±0.21	±0.05
21	2.37	3.94	0.23	9.19	11.83	10.17	8.61	18.49	0.78
	±0.36	±0.69	±0.07	±1.01	±1.30	±0.07	±0.17	±0.03	±0.03
28	.64	.74	-	3.64	4.73	-	1.19	1.45	-
	±0.23	±0.57		±1.28	±2.19		±0.07	±0.10	

^b In units of gram per denier

* All data at 95% confidence limit

* Reconstructed from Chu et al

The specimens at the physiological pH (7.4) retained the largest amount of breaking strength among the three buffers studied (Hac/NaAc, $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$, $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$). The observed pH –breaking strength retention profiles (figure 2.3.) from their study apparently suggested that the hydrolysis of the PLGA copolymer was catalyzed by either hydrogen (H^+) or hydroxyl ions (OH^-), as were many simple monomeric esters.

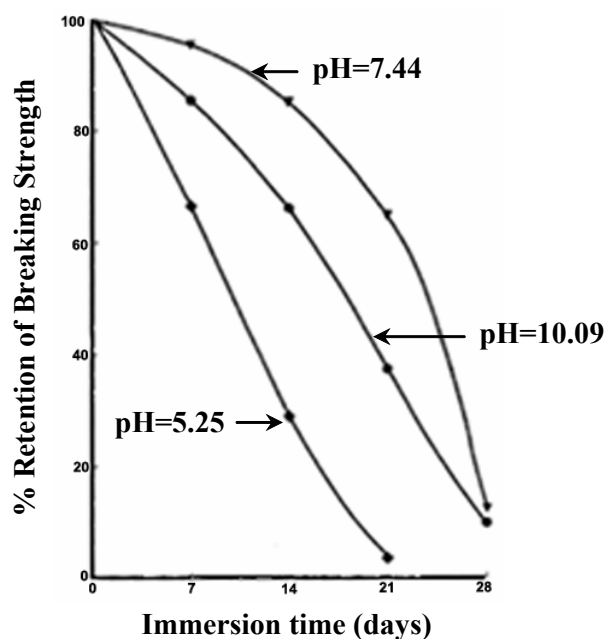


Figure 2. 3. Percentage retention of breaking strength of 2-0 Vicryl sutures at various extents of hydrolysis of three pH levels. ^[22]

In another study carried out in citrate-phosphate buffers at pH 5 and pH 7 and boric acid–borax buffer at pH 9 by Reed et al. ^[23] it was found that there was no significant change in degradation kinetics with change in pH. The results obtained in this study were not comparable with the results obtained by Chu et al. ^[22] who reported that PLGA copolymer degrades significantly faster in a highly alkaline buffer (pH=10.9) than in acidic (pH =5.25)

and physiological buffers (pH=7.44). The reason for the discrepancy could be that the composition and the physical form of the copolymer materials used by the two groups were different. The copolymer composition used by Chu et al. was 90/10-mol % glycolide/lactide and was in braided form whereas those used by Reed and coworkers were 50/50 and 70/30-mol % glycolide/lactide in the form of 1-cm diameter disc cut from 0.5 mm thick film, cast from CHCl_3 .

2.4.1.2. Effect of γ -irradiation on Hydrolytic Degradation of PLGA

In a study reported by Chu et al.^[24] it was shown that γ -irradiation treatment of polyglactin 910 sutures resulted in early appearance of the decreased pH region along the time scale on a typical pH vs. immersion time plot shown in figure 2.4. This was consistent with the observed faster loss of tensile strength due to γ -irradiation (figure 2.5.) observed in this study.

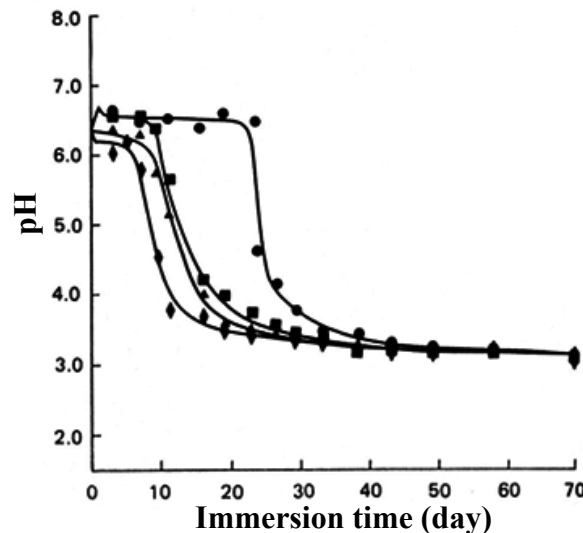


Figure 2. 4. Change in pH levels of poly (glycolide-lactide) braided fibers as a function of in vitro immersion time. (●) 0 Mrad, (■) 5 Mrad, (▲) 10 Mrad, (◆) 20 Mrad^[24]

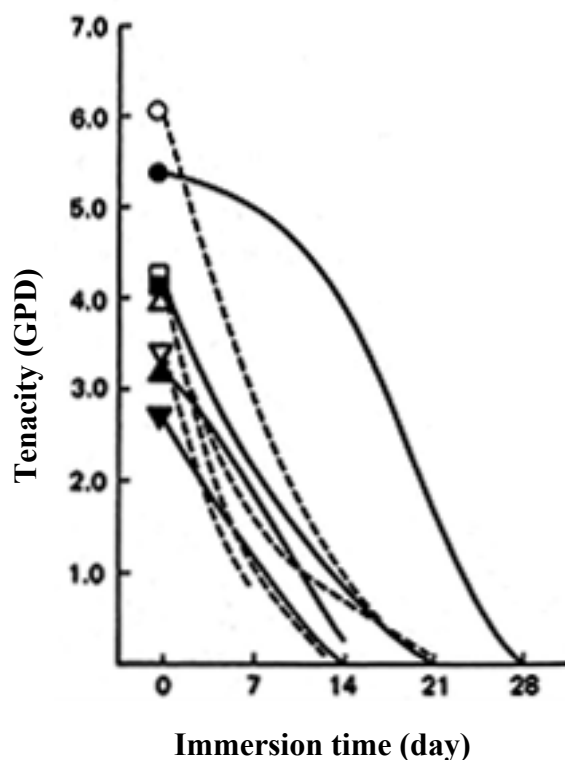


Figure 2. 5. Effect of γ -Irradiation on the change of tensile breaking strength of polyglycolic acid and poly (glycolide-lactide) braided fibers after predetermined period of immersion in buffer solution of pH= 7.4. (----) PGA; (—) PGL; (●) and (○): 0 Mrad; (■) and (□): 10 Mrad; (▲) and (△): 10 Mrad; (▼) and (▽): 20 Mrad²⁴

It was believed that γ -irradiation shortens the duration of the first stage of degradation through the preferential chain scission in the amorphous regions. Such a preferential fragmentation of the chain segment located in the amorphous region due to γ -irradiation would reduce the degree of chain entanglement in those regions and result in more open amorphous structure. Consequently, the irradiated specimen became more susceptible to hydrolysis and was reflected in the earlier appearance of the decreased pH region as well as the faster loss of tensile strength.

2.4.2. In *Vivo* Degradation of PLGA

2.4.2.1. Effect of Cellular Enzymes on Degradation of PLGA

Enzyme catalyzed enhancement of hydrolysis is one of the mechanisms by which a polymer can degrade in a living body. Since enzymes have the characteristic ability to catalyze certain chemical reactions, there is logic in the hypothesis that enzyme can accelerate *in vivo* polymer degradation. However in an investigation on polyglactin 910 sutures by Salthouse et al. ^[25] it was found that cellular enzymes do not play any role in absorption of this material. The experimental evidence from these studies summarized in table 2.7. demonstrates that the primary breakdown and absorption of polyglactin 910 sutures were independent of cellular or enzyme activity. The absorption rate data were identical for suture strands implanted solely in tissue or in a combination of saline solution incubation and *in vivo* implantation for similar total time spans, thus indicating that the only requirement for suture degradation and absorption was an aqueous environment. Salthouse et al. consider that the suture copolymer polyglactin 910 is degraded and absorbed through hydrolysis in the presence of water and that the only variable at body temperature is the length of time either in total *in vivo* treatment or in combination of *in vitro* and *in vivo* treatments. It was also evident from the study that even though hydrolytic enzymes play no apparent role in the initial hydrolysis of Polyglactin 910 sutures, cellular oxidative enzyme systems were active in the metabolism of the products of suture hydrolysis.

Table 2. 7. Comparison of Enzyme Activity, Methylene Blue Binding and Absorption at Polyglactin 910 Suture Implant Sites^[25]

Days			Acid Phosphatase [*]	Dehydrogenase [*]	Oxidase [*]	Methylene blue [†]	Percent Suture Remaining [♦]
A	B	C					
	7	7	±	+	+	±	100
	14	14	+	+	+	±	100
7	7	14	±	+	+	±	100
	21	21	+	+	+	±	100
7	14	21	+	+	+	±	100
14	7	21	±	+	+	±	100
	28	28	++	++	++	+++	100
7	21	28	++	++	++	+++	100
14	14	28	+	++	++	+++	100
21	7	28	±	++	++	+++	100
	35	35	++	+++	+++	+++	100
7	28	35	++	+++	+++	+++	100
14	21	35	+	+++	+++	+++	100
21	14	35	+	+++	+++	+++	100
	42	42	+	+++	+++	++	90
7	35	42	+	+++	+++	++	90
14	28	42	++	+++	+++	++	90
21	21	42	+	+++	+++	++	90
	56	56	+	+++	+	+	40
7	49	56	+	+	+	+	40
14	42	56	+	+	+	+	40
21	35	56	+	+	+	+	40

A, In vitro incubation 38 degrees C., pH 7.3

B, In vivo implantation.

C, Total time in aqueous environment, hydrolysis. In vitro plus in vivo.

* Enzyme activity grading: ± , trace activity; +, moderate activity, normal level; ++, increased activity; +++, intense activity. Acid phosphatase activity in macrophages at implant site. Dehydrogenase and cytochrome oxidase activity in cells adjacent to suture filaments.

[†] Methylene blue grading: ±, faint blue staining; +, pale blue staining; ++, blue staining; +++, deep blue staining. Methylene blue dye binding at pH 2.5 by suture filaments in cross section.

[♦] Suture absorption measured as percentage of cross sectional area remaining, estimated to nearest 10 percent

3. MATERIALS AND METHODS

To accomplish the objective of this work, an effective factorial design was developed. The experimental design was constructed to investigate simultaneously, the influences of material degradation characteristics, pick density and end density on the mechanical properties and porosity of the tubular structures. Five unique structures were thus constructed, which included all the combinations of structural variables.

3.1. Material Characterization

The material used for this study was a 56 denier-28 filaments Polyglactin 910 biodegradable yarn.

3.2.1. Filament Diameter

Individual filaments were cut at random from the yarn sample for filament diameter measurements. The diameter at 50 different places along the 5 specimens selected at random from the yarn sample was measured using a light microscope at a magnification of 400x.

3.2. 2.Yarn Linear Density

The linear density (yarn number) of the PLGA yarn sample was calculated according to the ASTM D1907-97 standard test method by the Skein Method. Five skeins of yarn were

wound and weighed. The yarn number was calculated from the mass and the length of the material by the following equation 3.1

$$YarnNumber(Denier) = \frac{Mass(gm)}{Lenght(m)} \times 9000 \quad (3.1)$$

3.2.Hydrolytic Degradation of Polyglactin 910 yarn

The nonsterile Polyglactin 910 yarn specimens were subjected to following degradation conditions for different time periods to determine the weight loss and tensile properties.

3.2.1. Weight Loss Determination

Approximately 5 m lengths of the PLGA yarn were used as the test specimens. The latter were weighed under standard laboratory conditions and placed in a Fisher brand clear glass screw capped vials containing 10 mL of buffer solution. The study was carried out over a period of 5 weeks in 0.01 M phosphate buffered saline solution of pH 7.4, at 37°C. In order to maintain the required pH of 7.4 the buffer solution was replenished with fresh buffer solution every 48 hours. Degradation was carried out in a thermostatically controlled orbital shaker water bath (BOEKAL Scientific, PA, USA). The solutions were maintained at 100 shakes/min. On removal from the incubator, all samples were washed with distilled water to remove residual buffer and dried over DRIERITE®.

Mass loss was determined for each sample by comparing the dry weight remaining at a specific time (m_t) with initial weight (m_o), where:

$$\% \text{ Mass Loss} = \frac{m_o - m_t}{m_o} \times 100 \quad (3.2)$$

3.2.2 Tensile Properties

Tensile testing was performed to determine the mechanical properties of the yarn and the effect of hydrolytic degradation on them. A special specimen-mounting frame was designed to mount the yarn specimens for degradation and tensile tests.

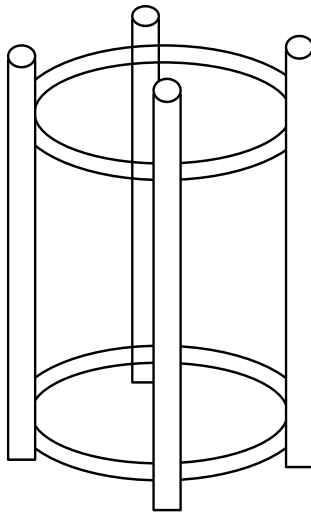


Figure 3. 1. Specimen mounting frame

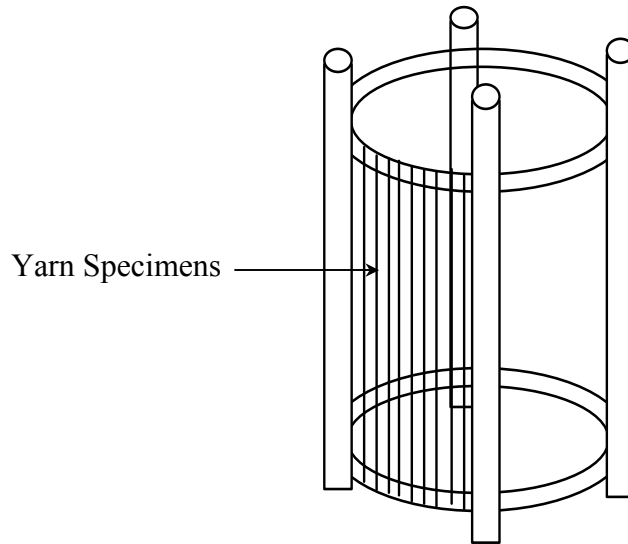


Figure 3. 2. Specimen mounting frame with yarn

The frame is made out of glass and is divided into four sections as shown in figure 3.1. Samples can be taken out from different sections after desired time period of degradation for

testing (figure 3.2). The frame fits in a Nalgene PMP 1000mL container, which can then be placed in desired degradation environment.

Approximately 35 in lengths of PLGA yarn were used as test specimens. Each test specimens was mounted in one section of the above-described specimen-mounting frame. The frame was placed in Nalgene PMP 1000mL container containing buffer solution. Degradation was carried out in a thermostatically controlled orbital shaker water bath (BOEKAL Scientific, PA, USA). The solutions were maintained at 100 shakes/min. The study was conducted over a period of 14 days in 0.01M phosphate buffered saline solution of pH 7.4 at 37°C and the samples were taken out after every 2 days. In order to maintain the required pH of 7.4 the buffer solution was replenished with fresh buffer solution every 48 hours. On removal from the incubator the samples were washed with distilled water to remove residual buffer and dried over DRIRITE®.

The tensile testes were performed on the Sintech® tensile tester under the standard atmospheric conditions. A 5-lb full-scale load cell was used. The gage length of the yarn was set at 5 inches. A yarn was clamped and stretched at a crosshead speed of 300 mm/min until the break occurred. The initial modulus, elongation at break, peak load and tenacity were calculated from the load elongation curve obtained from the machine. Five specimens for each yarn were tested for obtaining the average value.

3.3. Structural Variables

3.3.1 Number of warp yarns

The number of total ends used to weave the structure was varied in order to obtain grafts of different diameters as this was assumed to be the primary factor affecting the diameter. Three different number of ends were used; 38, 44 and 50.

3.3 2. Thread Densities

In order to develop structures with different porosities, thread densities, i.e. ends/inch (epi) and picks/inch (ppi) of the woven fabrics were varied. Weft or pick density was varied by changing the take-up roller speed on the loom. The pick densities used were 32, 38 and 44. These numbers represent ‘on-loom thread density’ i.e. the spacing between the yarns when the fabric is taut on the loom. The thread densities tend to change i.e. increase as fabric contracts on removal from the loom. Number 32 allowed more spacing among successive filling yarns while number 44 produced more dense structure along the warp. Warp or end density was changed by drawing different number of ends (or warp threads) through each dent of the reed. Two ends per dent pattern was used for this study.

3.3.3. Summary of the Structures constructed

The matrices shown in Figure 3.3 illustrate the 5 different structures produced. The vertical axis represents number of warp yarns (or ends) and the horizontal axis represents the

weft density (i.e. number of weft yarns per inch of the fabric). The first number in the combination is the number of warp yarns and the second is the weft density (e.g. 50/32 is the sample having 50 warp yarns and the weft density of 32).

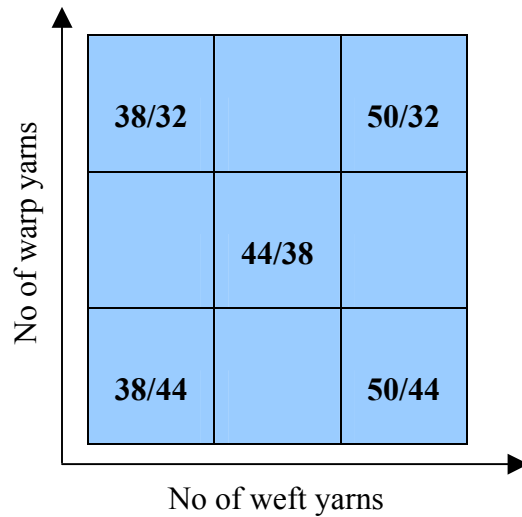


Figure 3. 3. Experimental design

3.4. Weaving Preparatory

The yarn obtained for this study was not in a suitable form to carry out weaving. For weaving to be performed on the yarn of the study certain preparatory processes were required to be used. These are described below.

3.4.1. Twisting

The original yarn obtained from the supplier was in a flat filament form, i.e., had no twist in it. Any weaving tried on this yarn led to fuzzing of filaments due to catching or

breaking of the individual filaments during the process. This occurs because the filaments fail to hold together and become separated (figure 3.4). The rubbing action caused by the reed weakened the filaments and the filaments broke over time. When sufficient number of filaments are broken, the yarn becomes weak and finally breaks. This caused the loom to stop. This occurred for multiple yarns at the same time and further weaving could not be possible until the warp sheet was freed from the broken filaments. If the weaving was started after clearing the filaments, problem re-emerged soon.

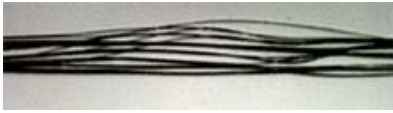


Figure 3. 4. Untwisted Yarn

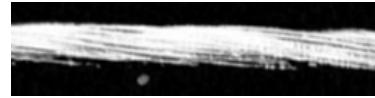


Figure 3. 5. Twisted yarn

To overcome this problem, the yarn was twisted with a ‘Z’ twist of 12 turns/inch to make the filaments adhere together (figure 3.5.). Twisting was carried out using ICBT two for one twister. The number of turns in a given length of yarn depends upon the spindle speed in revolutions per minute and take-up speed of the machine. The twist in the yarn on the machine is given by the following equation:

$$\text{Twist per meter (TPM)} = \frac{\text{Spindle RPM} \times 2}{\text{Takeup Speed(m/min)}} \quad (3.3)$$

3.4.2. Warping

After yarn was twisted, it was necessary to combine individual threads for further processing. This process is called "warping." Yarn packages from the twisting machine were placed on long metal frames called "creels." The yarn threads were pulled together into a single continuous strand and wound onto a metal beam called a "warper's beam." The number of threads in each strand of yarn and the warper's beam length are determined by the desired weaving construction. In the present study a total of 50 yarns were wrapped on a 'warper's beam' by using the warping machine shown in figure 3.6.

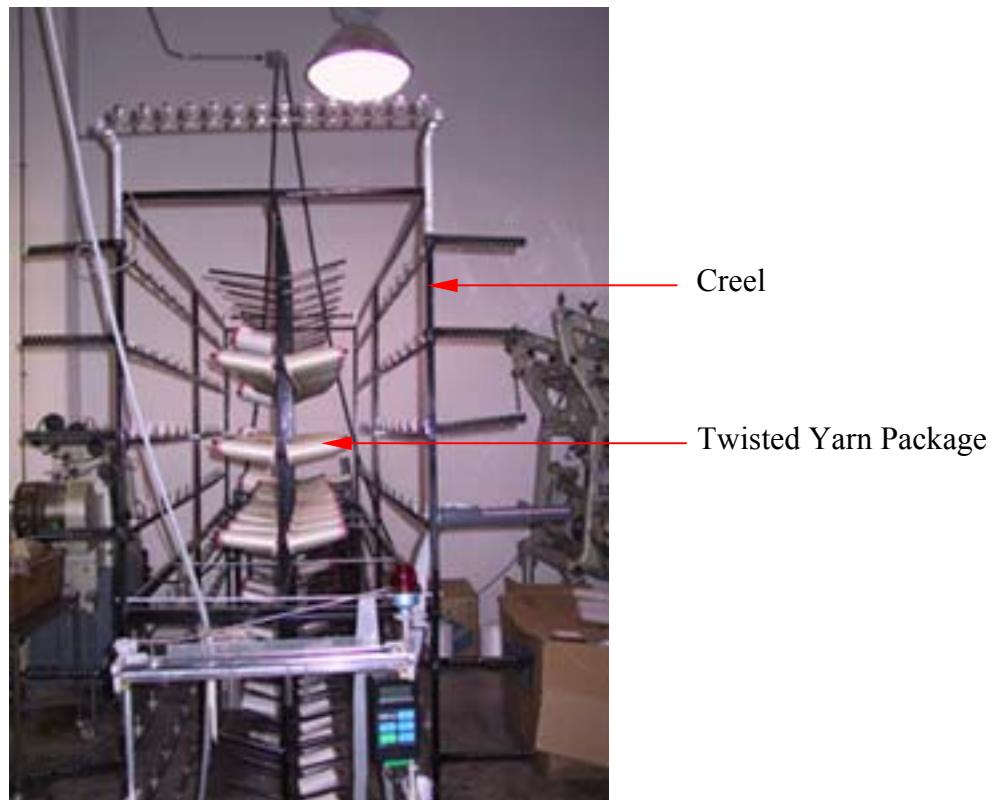


Figure 3. 6. Warping Machine

3.5. Weaving

3.5.1. Loom Used

The grafts were woven using a ‘Muller[®]’ loom (figure 3.7). The latter was fitted with Huttersley Dobby shedding motion with sixteen-harness capacity as shown in figure 3.6. This loom is particularly suited for preparing narrow width fabrics such as belts, tapes, and tubular fabrics.



Figure 3. 7. Loom used for weaving tubular structures

3.5.2. Weaving Particulars

A total of 50 yarns were wrapped on a ‘warper’s beam’ by using a warping machine discussed in 3.4.2. The beam was then mounted at the backside of the loom and warp yarns

were drawn through the ‘harnesses’. Eight harnesses were used and the warp yarns were drawn in a ‘straight draft’. These yarns were again drawn through the ‘reed’ at the front side of the loom. The reed used was 37-dent reed (37 dents per inch). The warp yarns were then wound around the ‘take-up roller’ that ensured the forward movement of the fabric as it was formed. Figure 3.8 shows the Draw-In-Draft (DID) and Chain Plan (CP) of the tubular plain weave.

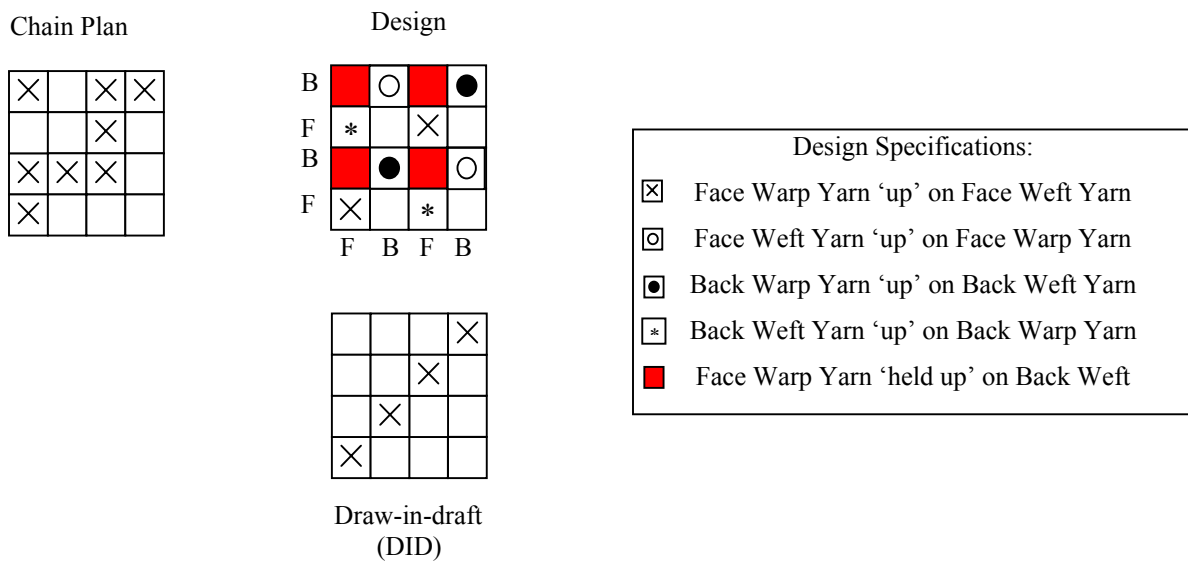


Figure 3. 8. Design, Chain Plan and Draft for the weaving of tubular fabric

3.5.3. Development of Tubular Structures

The basic fabric structure constitutes two sets of yarns, which are interlaced with each other in a specific pattern. One set of yarns (warp threads) runs longitudinally and the other (weft or picks) perpendicular to it, or cross-wise. The pattern was used was plain, in which one set of yarns run over and below the yarns in the other set alternately (Figure 3.9.)

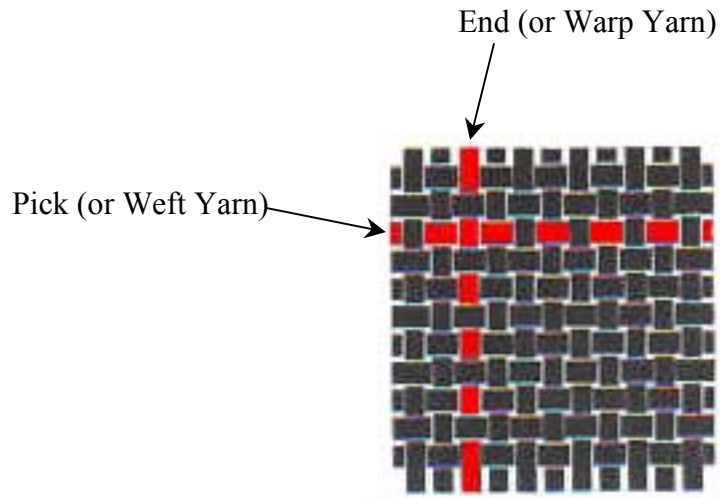


Figure 3. 9. Plain Weave Structure

The set up of the loom contains a 'beam', with the of warp yarns wound over it, a 'shuttle' that carries a bobbin over which the weft yarn is wound, the 'harnesses' that control the motion of the warp yarns (up/ down), a 'reed' that separates warp yarns and determines the spacing between them, and a 'take-up roller' that moves the fabric forward as it is formed. The motion of the harnesses is controlled by a mechanism called 'shedding' that is further controlled by 'pattern chain'. Thus the pattern chain, in effect, controls the weave design. The harnesses split the warp yarns into two layers during weaving. The reed determined the initial spacing between the warp yarns, or the end density (ends per inch, epi).

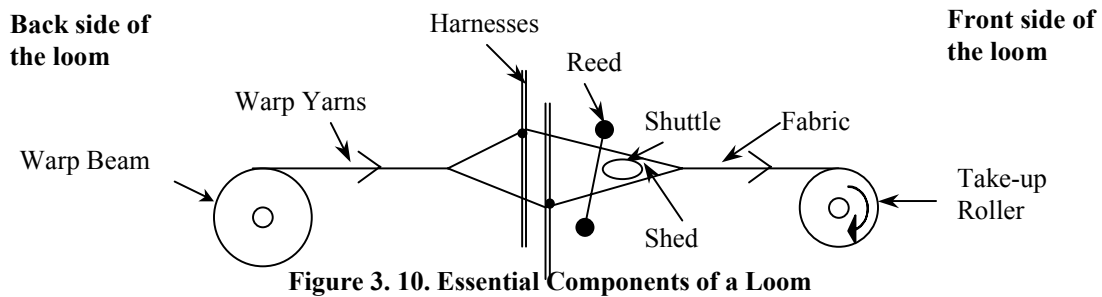


Figure 3. 10. Essential Components of a Loom

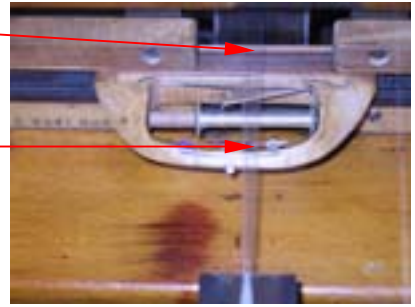
As weaving starts, half of the harnesses move up and the others down to form a ‘shed’ by splitting warp yarns into two layers (Figure 3.11 (B)). The shuttle with the filling yarn passes through it (Figure 3.11 (B)). and places a weft in the shed (Figure 3.11 (C)). Beating takes place at this stage to move the weft to the edge of the woven fabric. The two layers then cross each other and a new weft is placed again in the newly formed shed. Take-up roller turns each time the shed crosses and hence ensures the placement of weft yarn at different position along the warp and not on the top of the previous weft. The amount of turning of the take-up roller determines the spacing between successive weft yarns, determining the pick density.

A. Shuttle at the initial position



Weft Yarn

B. Shuttle passing through the shed



Warp Yarns

Shed

C. Shuttle after placing a fill yarn in the shed



Reed

Figure 3. 11. Different positions of shuttle in the loom

In case of the tubular fabric, the fabric itself is formed into two layers: the face or the top, layer and the bottom, or the back, layer.

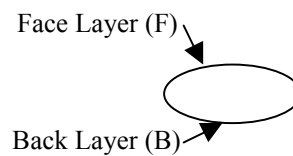


Figure 3. 12. Two separate layers to form a tube

As weft passes through the shed, it assists in the weaving of the face layer and on its way back it does the same for the back layer. When the shuttle places yarn in the shed for weaving of the back layer, the top layer (face layer) yarns are held up and are prevented from getting interlaced into the back layer. In this way, two layers of fabric are formed. The weft yarn used is continuous which weaves with the face layer and the back layer alternately and attaches these two at the edges to produce a tubular structure.

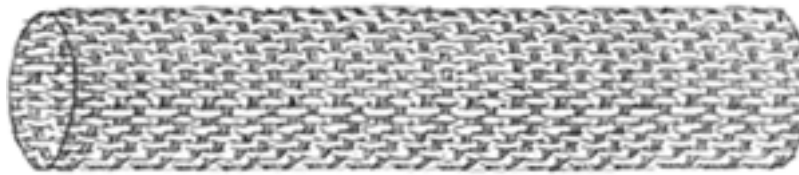


Figure 3. 13. Appearance of the Plain Woven tubular structure

3.6 Heat Setting of Tubular Structures

3.6.1. Identification of Heat setting conditions for Tubular Structures

The tubular structures removed from the loom after weaving were in a collapsed state. To make them round and resilient, heat setting was carried out.

To determine the ideal conditions for heat setting of the tubes, the yarns used for weaving tubes were heat set under a variety of conditions, that involved three different temperatures and three different dwell times. These yarns were then tested for tensile and elastic recovery properties to determine the setting conditions that gave optimum results.

The yarns were clamped on a specially designed frame, which allowed the clamping at various tensions. The tension used was set at 0.1g / denier that was sufficient to remove crimp and make the yarn straight. The yarns were heat set in the oven at three different temperatures namely, 125⁰C, 150⁰C, & 175⁰C. The duration, for which heat setting of each yarn was carried out, was also varied: 30 sec, 60 sec & 90 sec. Five specimens of each yarn were thus set at each of the combinations of temperature and time.

3.6.2. Yarn Tensile Testing

Yarn tensile testing was performed to determine the mechanical properties of the yarn and the effect of setting conditions on these. The testes were performed on the Sintech[®] tensile tester under the standard atmospheric conditions. A 5-lb full-scale load cell was used. The gage length of the yarn was set at 8 inches. A yarn was clamped and stretched at a crosshead speed of 300 mm/min until the break occurred. The initial modulus, elongation at break, peak load and tenacity were calculated from the load elongation curve obtained from the machine. Five specimens for each yarn were tested for obtaining the average value.

3.6.3. Elastic Recovery Determination

Elastic recovery test were performed on the yarn to determine the elastic recovery properties of the latter and the effect of setting conditions on these. The testes were performed according to the ASTM method D1777 for strain recovery of fibers. The tests were conducted on the Sintech[®] tensile tester under the standard atmospheric conditions. A 5-

lb full-scale load cell was used. The gage length of the yarn was set at 8 inches and three different strain levels from which recovery were determined were used. These were, 2%, 5% and 10%. Three specimens for each yarn were tested for obtaining the average value.

3.6.4. Heat Setting of Tubular Structures

From the tests on yarns, conditions were identified that were used for setting the tubes. The stainless steel rods whose circumference was approximately 80 - 90% of that of the tube were inserted into the tubes and secured on a steel frame. The frame was then passed through the oven preheated to a temperature of 150⁰C for a dwell time of 60 seconds (as determined from the studies on yarns). The tubes so obtained were final and evaluated for structural and mechanical properties.

3.7. Fabric Characterization

3.7.1. Pick density

Pick density was measured with 'pick glass', which was calibrated in inches. Pick density was measured three ways i.e. on-loom, off-loom before heat setting, and off-loom after heat setting.

3.7.2. End density

End density was calculated theoretically from the number of ends and the width of the collapsed and the heat set (in flat form) structures. A microscope equipped with a camera that projected the image on a monitor was used to assess the cross sectional dimensions. Having known the number of warp yarns used for the structure and the circumference, the spacing among the warp yarns (end density) was calculated and was expressed as ends per inch.

3.7.3. Diameter measurement

The widths of collapsed as well as heat-set (in flat form) structures were measured and it was assumed that the circumference of the tube was double the width of the tube in the flat state. The outer diameter of the tube was then calculated by dividing the circumference by π , or

$$D = 2W / \pi$$

Where, W = Width of the fabric in flat state

D = Outer Diameter of the tubular structure

3.7.4. Thickness measurement of Tubular Fabrics

The wall thickness of the tubular structures was measured by using a 'SDL Thickness Gage'. Three specimens each of area 1.5 cm² were cut carefully from each sample

and thickness measurement was carried out at different pressures. The graph of pressure Vs thickness was plotted and analyzed in order to estimate the thickness of the fabric at physiological pressures of 100 mmHg and 200 mmHg.

3.8. Weight loss determination of tubular fabrics

Approximately 2 cm lengths of heat set tubular fabrics were used as test specimens. Each test specimens was weighed and placed in a Fisher brand clear glass screw capped vials containing 10 mL of buffer solution. Non-heat set fabrics were used as control specimens. The study was carried out over a period of 4 weeks in 0.01M phosphate buffered saline solution of pH 7.4 at 37°C. In order to maintain the required pH of 7.4 the buffer solution was replenished with fresh buffer solution every 48 hours. Degradation was carried out in a thermostatically controlled orbital shaker water bath (BOEKAL Scientific, PA, USA). The solutions were maintained at 100 shakes/min. On removal from the incubation buffer all samples were washed with distilled water to remove residual buffer and dries over DRIRITE®.

Mass loss was determined for each sample by comparing the dry weight remaining at a specific time (m_t) with initial weight (m_o), where:

$$\% \text{ Mass Loss} = \frac{m_o - m_t}{m_o} \times 100 \quad (3.4)$$

3.9. Porosity

Porosity refers to the proportion of void spaces (pores) within the boundaries of a solid material, compared to its total volume. The porosity value, P , for the constructed tubular fabrics was calculated from the average mass per unit area of the fabric, M (g/cm^2), the average thickness of the fabric wall, L (cm), and density, ρ_m (g/cm^3), of the PLGA fibers, using the equation 3.5. The thickness of the fabric specimen were measured using a Digital SDL carpet thickness gauge (SDL international Ltd. Stockport, England) according to the procedure described in standard test method ISO 7198 1998(E) for cardiovascular implants-tubular vascular prosthesis^[27]

$$P = 100 \left[1 - \frac{M}{L\rho_m} \right] \quad (3.5)$$

3.10. Tensile testing of the tubular structures

3.10.1. Degradation of tubular structures

Approximately 4 cm in lengths of heat set and non-heat set tubular fabrics were used as test specimens. Each test specimen was placed in a Fisher brand clear glass screw capped vials containing 10 mL of buffer solution. The study was carried out over a period of 12 days in 0.01M phosphate buffered saline solution of pH 7.4 at 37°C and the samples were taken out after every two days. In order to maintain the required pH of 7.4 the buffer solution was

replenished with fresh buffer every 48 hours. Degradation was carried out in a thermostatically controlled orbital shaker water bath (BOEKAL Scientific, PA, USA). The solutions were maintained at 100 shakes/min. On removal from the incubation buffer all samples were washed with distilled water to remove residual buffer and dried over DRIRITE[®].

3.10.2. Specimen mounting for tensile testing

Tensile testing of the tubular structures was performed on the Sintech[®]. Load cell used was 5-lb. and the crosshead speed was set at 10-mm/min. A special frame designed by Moghe et al.^[26] was used to clamp the tubular structures on the Sintech[®]. The frame is shown in Figure 3.14.

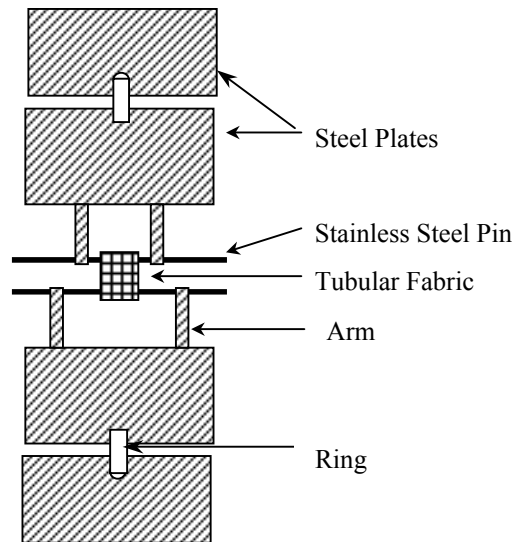


Figure 3. 14. Frame to clamp the tubular structures

The frame was constituted of two assemblies of steel plates: one for the upper jaw of the Sintech and the other for the lower jaw. Each assembly contained two steel plates interconnected by hinges or rings. This setting rendered the plates some freedom to move with respect to each other and it also ensured that the fabric sample would always be pulled vertically in a uniform fashion. Each assembly had two arms and each arm had one hole, of 1-mm diameter, at the end. Two stainless steel pins (one for each assembly) of diameter less than 1mm were inserted into the holes and the tubular fabric sample was clamped in between the two assemblies in such a way that it looped around the pins (Figure 3.15). The distance between the arms of one assembly was more than that between the arms of the other. This allowed two assemblies to come close to each other, while clamping the specimen, so that the tubes as small as 2 mm in diameter could be clamped and tested easily.

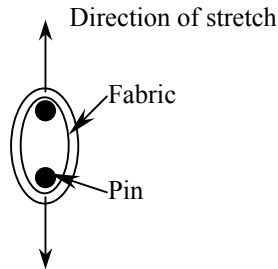


Figure 3. 15. Stretching of the fabric

3.10.3.Compliance Testing

For the compliance testing, five specimens from each sample were cut carefully to a width of 5 mm. As three different pick densities were used, the samples with different pick densities had different number of picks in the width of 5 mm.

The diameters of the structures were not the same and, therefore, the gage length of the fabric had to be determined for each tube. This was done by separating the jaws till a load of 1 gf was reached. This length was then considered to be the gage length. The fabric was stretched till the load developed in the fabric measured 50 gf. The data was digitally stored in the computer and a plot of load–elongation was developed for each sample. The slope of the curve was then determined by analyzing the load- elongation data over a range of pressures corresponding to 100-mmHg and 200-mmHg (13.3 kPa and 26.6 kPa). This is the physiological range of pressures that the grafts are subjected to when used as replacements for blood vessels. The inverse of the slope is the compliance of the structure for the physiological range of pressure.

Compliance = strain/ stress = 1/ initial modulus of the tube

3.10.4. Elastic Recovery testing

Elastic recovery test was also carried out on the Sintech[®] tensile tester. 10 mm wide samples were cut for this purpose. The frame used to clamp the fabric is shown earlier in Figure 3.14.

The fabric was elongated, at a speed of 10 mm/min and as shown in Figure 3.14, till the load measured 50gf. The gage length (length at 1 gf load) was recorded as the initial length for the first cycle. The cross-head was raised and fabric was elongated again until the load developed was 50 gf. The cross-head was then lowered until the load dropped to 2 gf. This was the approximate value of load corresponding to 100 mmHg for all the structures. The sample was allowed to recover for 10 sec and then stretched again to the previous level (50 gf). This procedure was repeated 5 times. The data representing the load-elongation behaviors of the structures was digitally stored from which the graph of load versus elongation was developed. The elongation at 2 gm load was subtracted from the elongation at 50 gm load, for each cycle, in order to obtain the recovered elongation. The percentage of this value relative to the total elongation for that cycle gave the elastic recovery of the structure.

4. RESULTS AND DISCUSSION

4.1 Effect of Hydrolytic Degradation on Properties of PLGA Yarn.

A systematic study of the changes in behavior of PLGA yarn after hydrolytic degradation was conducted. The properties examined were the changes in yarn mass and tensile properties, and transverse dimensions under an optical microscope. The results obtained provided some understanding of what happened to the yarn and allowed us to establish a basis for rationalizing the changes that took place in the properties of the grafts woven from these yarns.

4.1.1. Change in Mass of PLGA Yarns due to Hydrolytic Degradation

The percentage mass loss calculated relative to the initial dry mass of the PLGA yarn specimens gave an understanding of the degree of the hydrolytic degradation of this material. Table 4.2. gives the percent mass loss at different time periods. Figure 4.1 gives a plot for these results. It is seen from the results that there is no significant change in mass upto 7 days. Statistical t-test conducted at 95% confidence level confirmed this (Table 4.1.). The loss, however, is significant with continued degradation, being 11% after 14 days and 50% after 28 days.

Table 4. 1. Statistical Analysis for Mass Loss of PLGA Yarns

No of days	Mean Weight (g)	SD	t Calculated	t Critical (95% Confidence Level)	P Value
0	0.0315	0.0020	0.011	2.78	0.91
7	0.0314	0.0020			

Table 4. 2.Mass Loss data of PLGA 56-Denier Yarns

Days	% Weight Loss
7	0.64(±0.04)
14	11.10(±0.91)
21	32.06(±0.92)
28	52.20(±0.80)

The mean values are averages of 3 specimens. Given after the mean value is the value of 1 standard deviation

As seen from the plot (Figure 4.1), the mass loss is exponential suggesting bulk hydrolysis of the polymer material. The data fitted exceptionally well on the following exponential model:

$$\Delta W = aT^n \quad (4.1)$$

where, ΔW is percent change in mass

T is time in days

a and n are constants whose values were found to be 0.0015 and 3.2385 respectively

The data fitted on this model gave the R^2 value of 0.97. This model is plotted in figure 4.1, which shows excellent correlation with the experimental value.

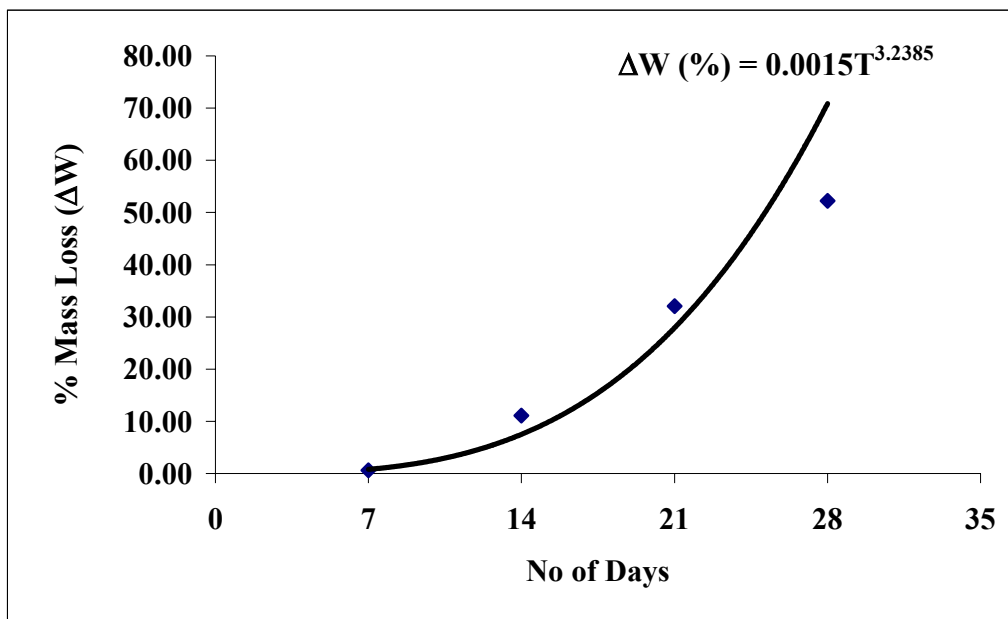


Figure 4. 1 Mass Loss of PLGA 56 Denier Yarns upon Hydrolytic Degradation

4.1.2. Change in Tensile Properties Due to Hydrolytic Degradation

The mechanical properties of the PLGA yarns subjected to degradation for different number days were assessed. The individual load-elongation curves obtained are put in Appendix 1. Figure 4.2 shows the representative stress –strain curves for different times of degradation. The mean values of tensile properties assessed are presented in Table 4.3.

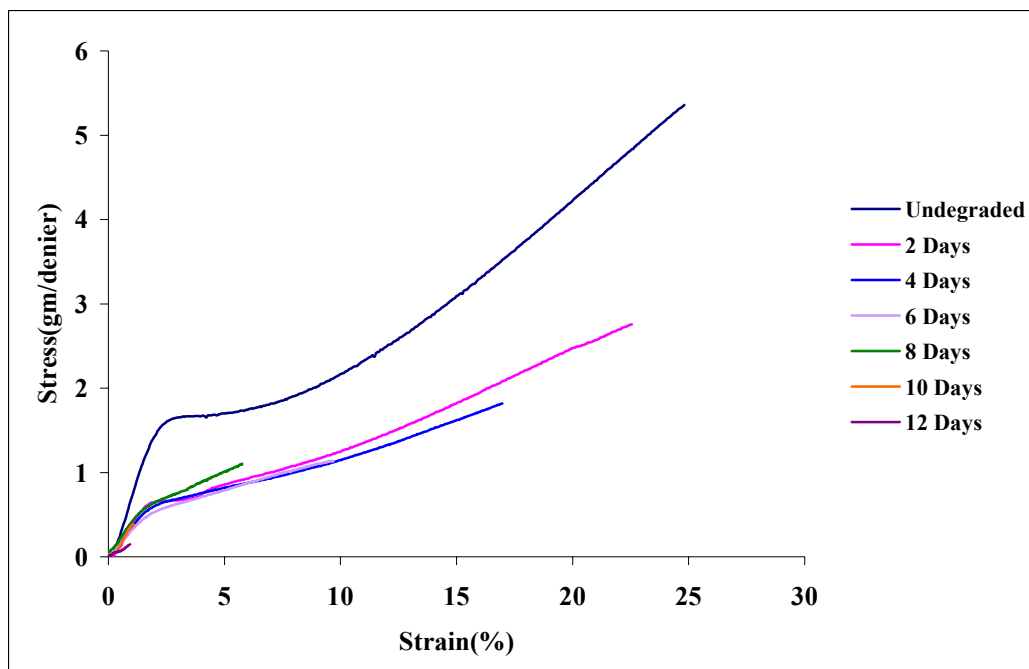
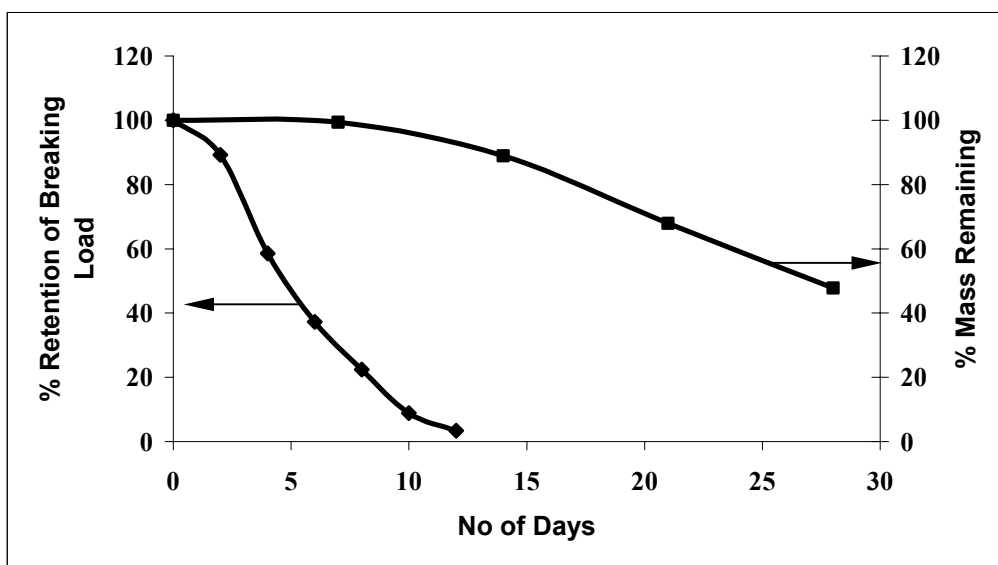


Figure 4. 2 Representative Stress –Strain Curves for Different Time of Degradation



8	69.83±1.60	5.53±0.26	36.78±2.19
10	27.56±2.38	1.41±0.09	25.63±3.58
12	10.52*	1.19*	13.42*

The mean values are averages of 4 specimens. Given after the mean value is the value of 1 standard deviation

*Based on only one specimen

Figure 4.3. Shows a plot of percent breaking strength retained as a function of number of days. It is seen that the loss of tensile strength is rapid and that load-bearing properties have declined to about zero in two weeks. For comparison, percent mass retained is also plotted in this Figure 4.3. It is seen that the mass loss is not comparable to strength loss but lags behind, indicating bulk erosion of the polymer material.^[27] Diffusion of water molecules into amorphous domains probably resulted in scission of taught tie molecules that connected the crystalline regions. Fifty percent strength loss occurred practically without any loss in mass. That the mass decreased slowly is also obvious from the optical micrographs included later in section 4.1.3.

Figure 4. 3 Percentage of Retention of Breaking Strength and Mass of PLGA 56 Denier Yarns upon Hydrolytic Degradation

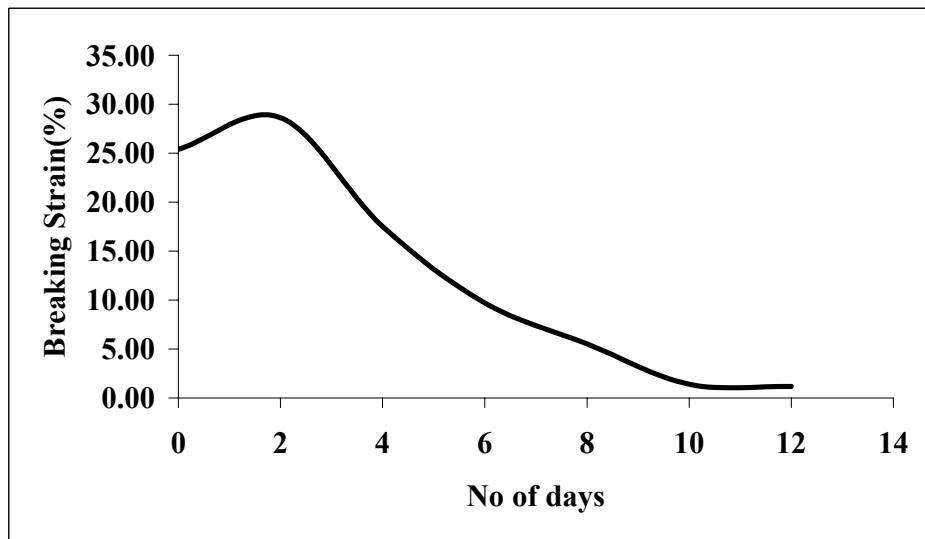


Figure 4. 4 Changes in Breaking Strain Upon Hydrolytic Degradation

The effect of number of days of degradation on initial modulus is illustrated in Figure 4.5. The initial modulus of the material is given by the slope of the stress strain curve at origin, and is a measure of the resistance to elongation before yield point. The reciprocal of this property is compliance, which is one of the key parameters that determine success of a small diameter graft in bypass application.

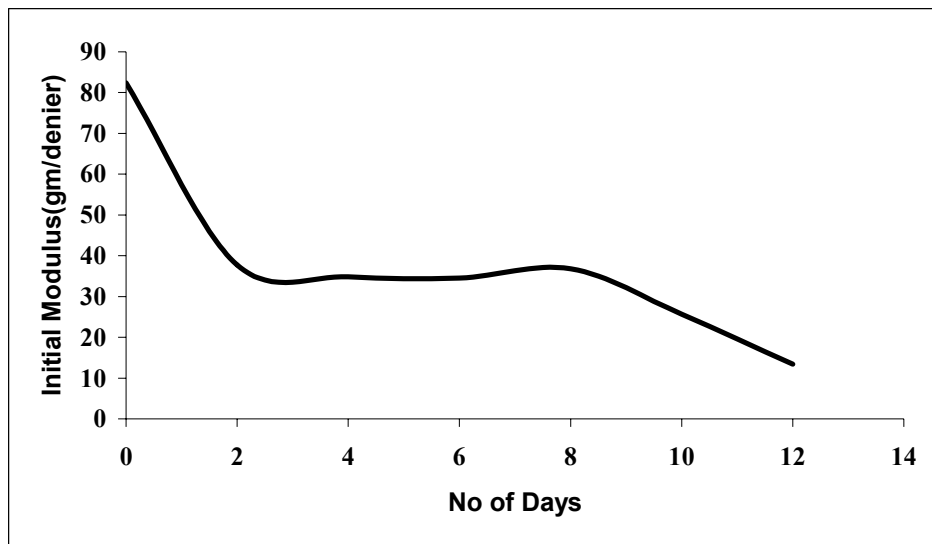


Figure 4. 5 Initial Modulus of 56 Denier PLGA Yarn after Hydrolytic Degradation

From the plot it is observed that the modulus drops by fifty percent within the first 48 hours of degradation. It then stays fairly uniform for the next six days where as the breaking strength continues to decrease over the entire period. The modulus again drops rapidly after eight days and is about only 16 % of the initial value after 12 days. Weight at the end of 12 days was over 90 % of the initial value, i.e., the material had only lost about 10 % of the weight by then. Some of the changes noted above in mass and properties can be understood by examining the fiber under a polarizing microscope, as done in the next section.

4.1.3 Observation of Surface Morphology and Transverse Dimension under an Optical Microscope.

Longitudinal views of PLGA filaments degraded for different time periods were examined using a polarized light microscope. Filament diameter was measured using a stage micrometer under 400X magnification. Table 4.4. gives the measured values of filament diameter after degradation at different time periods. The images obtained are shown in Figure 4.6.

From the images it is seen that no visible surface features (such as cracks) are detected on the undegraded specimen (0 days). A filament's surface however started to become rough around 4 days of immersion. At day 14, the filaments were observed to have formed transverse pits (cracks) across the cross section. These changes correspond to almost the complete loss in strength noted earlier after a two week period. Interestingly, the filament diameter did not change during this same period. Statistical analysis conducted showed that

the change over the 14 days was highly insignificant. The values given in Table 4.4. are averages of 50 readings taken on 5 specimens.

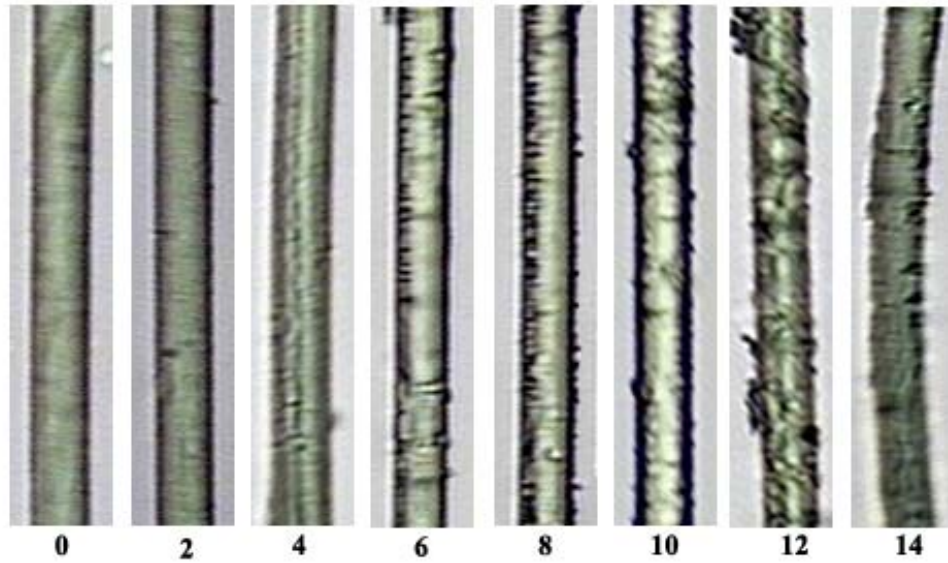


Figure 4. 6 Longitudinal Views of Undegraded and Degraded PLGA Filaments (Magnification 400X)

Table 4. 4 Filament Diameter

Degradation Time (Days)	Diameter (μm)
0	15.00
2	15.00
4	15.00
6	15.00
8	15.00
10	15.00
12	15.00
14	14.75

4.1.4 Discussion of Changes in The Yarn Properties

Tensile studies conducted on the undegraded and degraded yarns showed that the peak load decreased with increase in duration of immersion time where as the breaking strain increased initially after two days degradation and then decreased with further increase in immersion time. This may be because water molecules that diffuse through the polymer act as a plasticizer material rendering the chain molecules more flexibility to move by a lubrication action. However, with increase in immersion time the chains get degraded and break into fragments, which led to decrease in breaking strain. The strength of the material is the measure of the weakest point present within it. With increase in immersion time, more and more tie chain segments and chain folds degrade into fragment increasing the total number of weak points in a given yarn segment, which increased the probability of such material breaking at lower loads.

It is observed from the tensile tests data (Figure 4.5) that initial modulus does not follow the same trend as that of peak load. The latter decreased with increased immersion time, where as initial modulus decreased rapidly after 2 days of degradation and then stayed uniform for next 6 days and finally decreased further. This could be because initial modulus is not the result of ultimate changes that took place in the yarn. Since it is given by the slope of the stress-strain curve at origin, it is not governed by the ultimate load that a material can sustain but by the loads that affect the initial portion of curve. It is possible that there is a rapid change in the chain molecules during the initial phase of degradation and then they get stabilized thus giving a same value of initial modulus after 2 days of immersion time.

4.2. Effect of Heat Setting on Yarn Properties

It was pointed out in the section 3.6 that the heat setting process was necessary to make the flattened tubular structures round and resilient. Perfectly round tubular structures are essential to avoid the blood flow disturbances when the graft is used as replacement for blood vessels. Also, for long-term patency, the optimum mechanical properties possible in a given material are desirable. Given heat setting process is essential for developing circular resilient structures and that such process affects mechanical properties, it was necessary to examine the effect heat setting conditions had on yarn properties. This work was conducted on yarn was necessary as it helped in identifying optimum conditions one may use for heat setting tubular grafts.

Yarns were heat set using three different temperatures and three different dwell times as discussed in section 3.6.1. These parameters chosen for experiment were based on our knowledge of the conditions used by industry in heat setting and texturizing synthetic materials. The mechanical properties of the yarns were assessed after they were heat set. The values of tensile properties assessed from these are presented in Table 4.5.

Table 4. 5. Effect of Heat setting Conditions on Mechanical Properties of Yarn

Temp (°C) and Time (sec)	Peak Load (gm)	Tenacity (gf/Den)	Strain @ Peak Load (%)	Initial Modulus (gf/Den)
Non heat set	311.20±7.80	5.94±0.17	25.40±0.68	81.46±5.32
125/30	338.59±5.02	6.05±0.09	26.54±.84	71.88±1.68
125/60	343.97±10.15	6.14±0.18	26.36±0.81	74.42±2.32
125/90	345.89±15.57	6.18±0.28	23.77±0.87	77.47±3.36
150/30	309.29±8.89	5.52±0.16	24.41±1.74	72.56±2.33
150/60	341.47±9.09	6.10±0.16	27.02±0.63	68.26±1.52
150/90	326.53±10.81	5.83±0.19	21.42±0.92	75.74±2.91
175/30	272.84±12.46	4.87±0.22	22.83±1.25	68.84±1.09
175/60	261.77±6.01	4.67±0.11	22.44±0.70	67.48±0.74
175/90	267.98±8.17	4.79±0.15	18.36±0.85	71.77±1.09

The mean values are averages of 5 specimens. Given after the mean value is the value of 1 standard deviation

4.2.1. General Effect of Heat Setting Temperature and Dwell Time on Tensile Properties

It was observed that the temperature and dwell time had significant effects on the peak load (and tenacity) of the yarn (Figures 4.7). This was confirmed by conducting the student t-test at 95% confidence level. The peak load increased slightly as temperature increased from ambient to 125⁰C. Beyond this, an adverse effect on peak load occurred for 30 and 90 sec dwell time. For 60 sec specimen, this change occurred at 150⁰C. The dwell time showed significant effect on peak load at the two setting temperatures (125°C and 150°C) used. Peak load was highest for the specimen set at 125°C for 90-second dwell time.

The modulus of the yarn decreased almost in a linear fashion till temperature of 125°C (Figure 4.8). After this temperature, the change is small (< 2%) except in the case of sec dwell time where the change is about 8 %. The effect of dwell time was also definitive,

modulus increased with dwell time at 125°C. At 150°C, the value was highest for 90 sec and lowest for 60 sec dwell time.

The peak strain values are reasonable constant except when the temperature is 175°C or the temperature dwell time combination is 150°C/90 sec. Under the latter conditions the peak strain values are significantly lower. From these results one may narrow down the choices for setting of tubular structures. The final criteria to be used will be the modulus and elastic recovery properties, discussed in the next section.

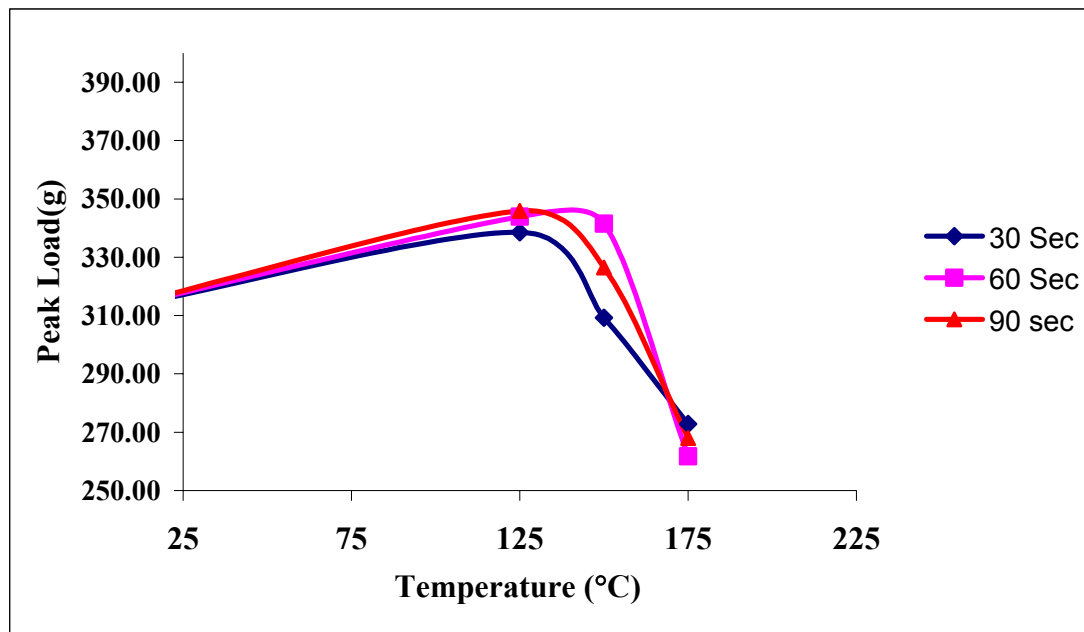


Figure 4. 7 Effect of Heat Setting Temperature and Duration on Peak Load

4.2.2. Effect of selected Heat-setting conditions Modulus and Elastic Recovery Properties

After the initial screening of the tensile data, the choices for heat setting conditions were narrowed down to four namely, 125°C /60sec, 125°C /90sec, 150°C /30sec, 150°C/60sec. These were selected based on values of tenacity and breaking strain and an understanding of the effects setting conditions produced on structure and properties. To narrow down the choices further i.e., to one set of conditions, the initial modulus and elastic recovery properties of the heat set yarns were assessed. Table 4.6. shows the modulus and elastic recovery results for the yarn set at these conditions.

The initial modulus of the yarn is an important property because it directly affects the compliance of the tube, the latter being the inverse of the modulus. The modulus was assessed by analyzing the load-elongation data of the yarn. A lower modulus gives higher compliance which is preferred in graft application.

From the elastic recovery values one sees that for same dwell time the elastic recovery is slightly higher for higher temperatures. This is true at all strain levels. Within the same temperature zone a larger dwell time gave a larger elastic recovery.

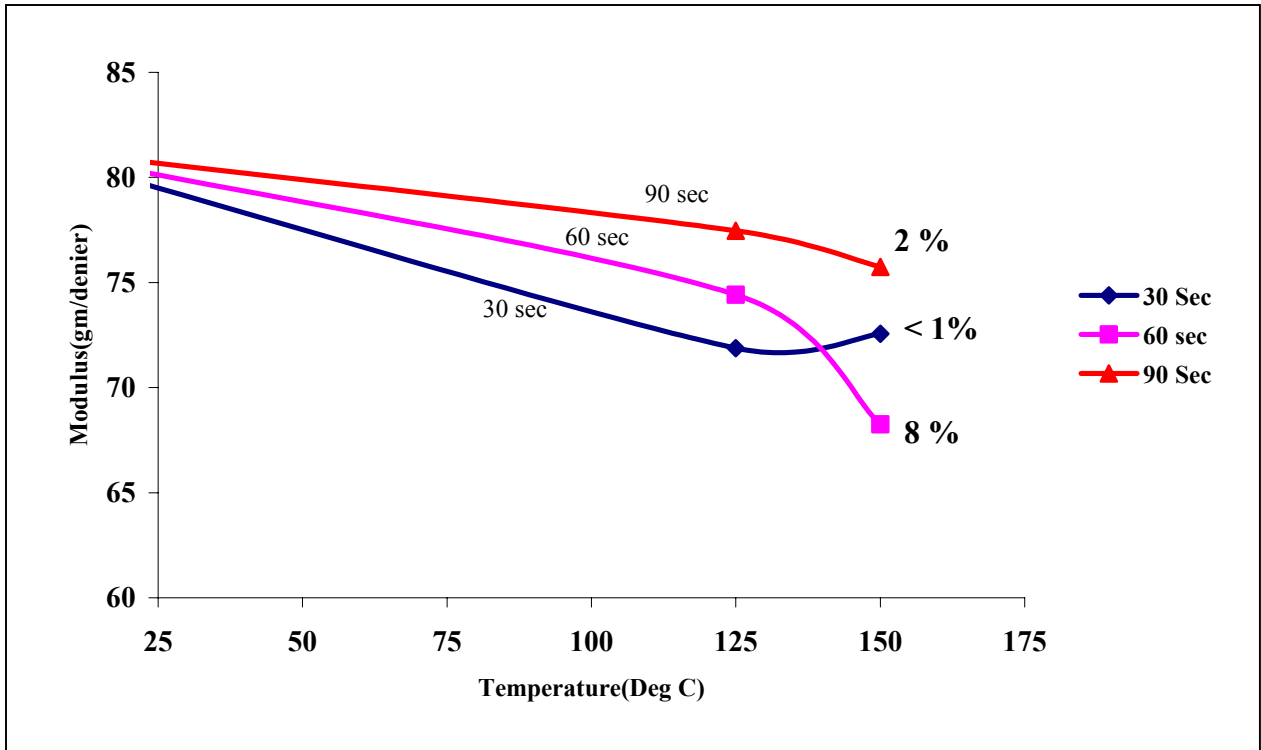


Figure 4. 8 Effect of Heat setting temperature and duration on Modulus

Table 4. 6. Effect of Heat Setting on Elastic Recovery of Yarn

Temp and Time	Initial Modulus (gf/Den)	Elastic Recovery (%)		
		2 (%) strain	5 (%) strain	10 (%) strain
125/60	74.42±2.32	79.92	74.70	65.83
125/90	77.47±3.36	82.75	75.97	65.68
150/30	72.56±2.33	80.83	75.26	65.83
150/60	68.26±1.52	81.83	76.20	66.72

4.2.3. Conditions Selected for Heat Setting of Tubular Structures

Table 4.5 and 4.6. show all the mechanical properties assessed after the treatment of yarns at different temperatures and dwell times. The temperature and dwell time chosen for heat setting of tubular structures were 150⁰C and 60 seconds respectively. These conditions offered the best combination of mechanical properties in terms of the peak load, modulus and elongation at peak load and elastic recovery.

4.3. Characterization of Woven Grafts Before and After Heat Setting

As has been seen earlier, heating setting of the polymeric material has profound effect on the properties of these materials. The heat setting treatments thus, should also affect the mechanical behavior and structural properties of products made from such materials. So it is very important to determine what changes in properties, heat treatments produced on the products. The effects of the heat setting treatment on the structure and properties of the woven grafts were determined. The results presented and discussed are in the following sections.

4.3.1. Effect of Setting on Pick and End Density

The pick density is one of the important parameters of the tubular fabrics that control the properties of the structure in transverse or the filling yarn direction. Pick density determines the number of yarns present in a given width of the fabric. As the load distribution depends on the number of yarns present, pick density is a determining factor for mechanical properties of the grafts subjected to forces in the transverse direction.

The values of pick density were measured on the tubes under three different states: on-loom, off-loom and off-loom after heat setting. The measurements were conducted with the help of a 'pick glass'. Table 4.7. gives the results.

Table 4. 7. On-loom, Off-loom and Post-heat-setting Pick Densities of the tubular structures
(Picks per inch)

Sample (no. of ends/ pick density)	On-loom	Off-loom	Post Heat-setting
50/44	44	47	46
50/32	32	34	34
44/38	38	41	41
38/44	44	46	46
38/32	32	34	34

From the results it is seen that the pick density tends to increase when the fabric is removed from the loom. On the loom, the warp yarns are under tension, which is applied to the warps for proper weaving action and adjusted in such a way that it is always within the elastic limit of the material, i.e. it does not cause significant elongation in the threads. Essentially, the tension applied takes up all the crimp from the yarn. On removal from the loom, the yarns tend to reacquire their crimped state and so contract length-wise. The contraction makes the filling yarns come closer, which consequently results in an increase in pick density. During heat setting, it is observed that the structures shrank only width-wise (not length wise) and, therefore, virtually no change took place in pick density.

End densities were calculated from the calculated values of tube diameters and number of warp yarns. The calculated values of end density before heat setting and after heat setting are listed in Table 4.8. The heat set fabrics have higher values. This observation is in

corresponding with the comment made above, i.e. the structure shrank width wise. This means, the diameter of the tube decreased and, therefore, the end density increased.

Table 4. 8.End Densities of Structures Before and After Heat Setting (Ends/Inch)

Sample (no. of ends/ pick density	As Woven	Heat-set
50/44	57.31	63.00
50/32	56.60	62.01
44/38	78.48	83.40
38/44	88.07	98.49
38/32	70.15	74.02

4.3.2. Diameter of Tubular Structures

As mentioned earlier, the diameters of the rods selected for heat setting of fabrics were 80% to 90% of the diameters of the woven tubes. This allowed all fabrics to shrink down and acquire the circular shape of the rod. This shape was then ‘set’ due to thermal energy. Accordingly, the diameters of the grafts were changed during the setting process. The final diameters of the structures produced are given in Table 4.9.

Table 4. 9. Diameters of tubular structures before and after heat setting (mm)

Sample (no. of ends/ pick density)	As Woven	Heat-set	% Shrinkage
50/44	7.06±0.386	6.42±0.095	9.031
50/38	7.15±0.276	6.52±0.132	8.73
44/38	4.54±0.209	4.27±0.127	5.90
38/44	3.49±0.276	3.12±0.135	10.58
38/32	4.38±0.228	4.15±0.145	5.23

The mean values are averages of 5 specimens. Given after the mean value is the value of 1 standard deviation

The number of warp yarns used was one of the primary factors that determined the diameter of the tubular structures. It had a non-linear but direct effect on the diameter value, the more the number of warp yarns, the larger the diameter of the tube and vice versa. The results in Figures 4.9 show that, this relation holds true for all structures except one, i.e. 38/32. A higher diameter than expected in this case may be due to the possibility that the pick density is below certain value so that the warp tension used caused the warp yarns to be less crimped or straighter. One also observes that for same number of ends, greater pick density leads to smaller diameter. This is expected due to the fact that a pick pulls warp yarns closer and, therefore, greater the pick density, smaller should be the diameter. This explanation also supports the departure noted earlier.

The diameters of the heat set structures are the values considered in the evaluation of the behaviors of the grafts in the sections that follow.

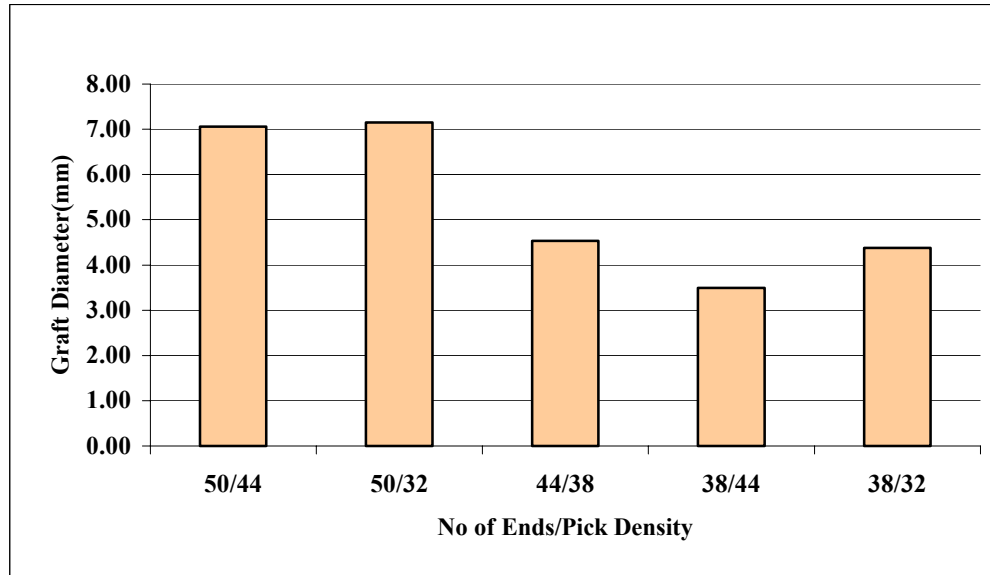


Figure 4. 9 Effect of Number of Warp Yarns (ends) on Diameter of the Structures

4.4. Properties of Tubular Structures

One of the primary objectives in this research was to examine and understand the degradation behavior of the grafts woven from PLGA yarns and evaluates their effects and the construction parameter on the mechanical properties. After adequate heat setting of the woven structures obtained from the loom, an array of tests were performed. The variables were the construction parameters and the days of degradation; and the properties examined were the change in mass, and thickness of the graft, and the elastic recovery, compliance and porosity properties of the structures.

4.4.1. Change in Mass of Tubular Fabrics upon Hydrolytic Degradation

The heat set grafts were subjected to degradation conditions following the procedure described in section 3.8. Values of the percentage mass loss of the fabrics were determined and are presented in Tables 4.10.

Table 4. 10. Mass Loss Data of Tubular Structures

Sample (no. of ends/ pick density)	% Mass Loss	Sample (no. of ends/ pick density)	% Mass Loss
7 Days		21 Days	
50/44	0.51± 0.44	50/44	30.98± 1.30
50/32	0.84± 0.85	50/32	31.40± 1.42
44/38	0.69± 0.60	44/38	30.50± 1.87
38/44	0.73± 0.63	38/44	33.59± 0.76
38/32	1.11± 1.11	38/32	31.36± 1.30
14 Days		28 Days	
50/44	11.67± 0.31	50/44	48.76± 3.03
50/32	11.75 ± 0.85	50/32	51.48± 2.09
44/38	12.40± 0.65	44/38	49.73± 2.69
38/44	11.94± 0.71	38/44	51.41± 3.43
38/32	12.17± 0.77	38/32	48.45± 3.03

The mean values are averages of 3 specimens. Given after the mean value is the value of 1 standard deviation

The data indicates that the number of ends and picks do not have an effect on weight loss. Statistical analysis confirmed this observation. The weight loss, thus, is strictly the characteristic of the polymer material. To confirm this conclusion a plot between percent mass loss and degradation time was obtained for three constructions varying in terms of both the number of ends and picks. The plot is compared with the predicted curves for polymer

obtained from equation 4.1. The mass loss values for the three different constructions overlap each other and together fit closely to the predicted curve. This indicates that the mass loss is only influenced by the polymer properties and not to any extent by the fabric construction parameters.

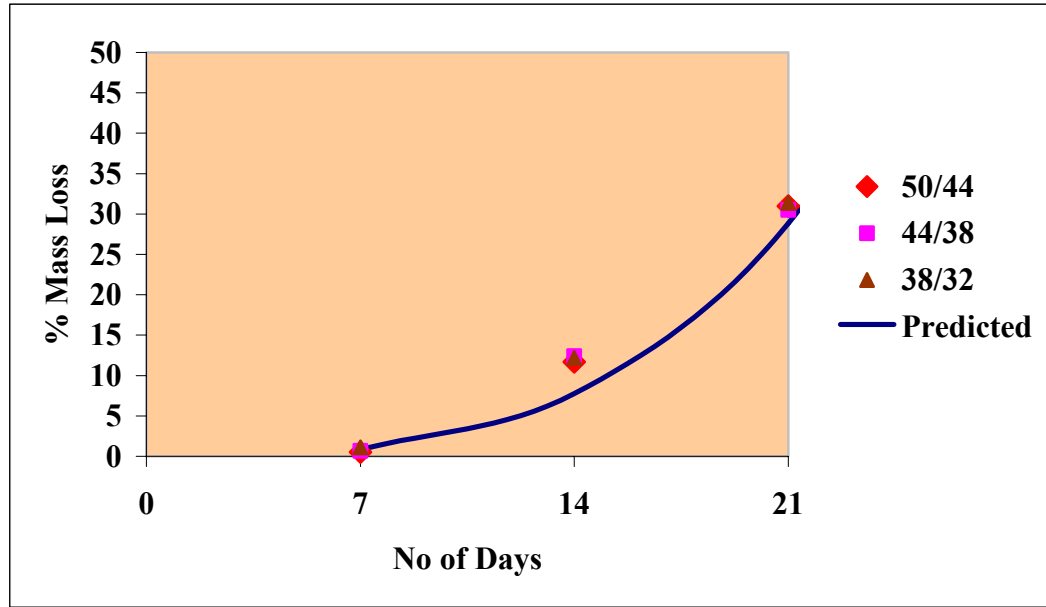


Figure 4. 10 Effect of no of Ends and Pick Density on Weight Loss

4.4.2. Thickness

Thickness of the tubular structures can be considered as another important factor that affects the mechanical properties of the grafts. This is because thickness affects the area of the fabric over which the physiological pressure applied is distributed. The greater the thickness, the lower is the compliance of the fabric at the same pressure.

It is understood that the thickness of a fabric is a function of the pressure used during measurement. Typical pressure-thickness relationship is exponential in character i.e., with increase in pressure the thickness decreases rapidly initially and approaches an equilibrium value after which there is no further change with increase in pressure. Taking into account this fact, thickness measurements were made at two different pressures, namely, 100 mmHg (13.3 kPa) and 200 mmHg (26.6 kPa). These are the typical values of pressure used in biomedical research to represent the physiological conditions in the *in vitro* tests of medical devices. Table 4.11. summarizes the measured values of the thickness of the grafts.

Table 4. 11. Thickness values of tubular structures (mm)

Sample (no. of ends/ pick density)	Pressure (mmHg)	Number of Days of Degradation						
		0	2	4	6	8	10	12
50/32	100	0.11	0.11	0.11	0.11	0.11	0.11	0.10
	200	0.10	0.09	0.09	0.09	0.09	0.09	0.09
50/44	100	0.12	0.12	0.11	0.11	0.11	0.11	0.10
	200	0.11	0.10	0.09	0.08	0.08	0.09	0.08
44/38	100	0.12	0.12	0.11	0.12	0.12	0.11	0.12
	200	0.10	0.10	0.09	0.10	0.11	0.10	0.10
38/32	100	0.12	0.12	0.12	0.12	0.11	0.11	0.11
	200	0.11	0.10	0.11	0.10	0.10	0.10	0.09
38/44	100	0.12	0.12	0.12	0.12	0.12	0.11	0.12
	200	0.11	0.10	0.09	0.11	0.10	0.09	0.10

Calculation of thickness values is shown in Appendix

The results in table 4.11 show that, as expected, with an increase in pressure from 100 mmHg to 200 mmHg, the thickness of the fabric decreased. In the undegraded material this decrease is about 10%. With degradation, the change is noted to be greater, on an average being approximately 20%. Assuming degradation caused breakages in cross-links, this

should result in the structure being somewhat more compressible. Upon comparing the thickness values statistically, it is found that there is no significant difference between the thickness values at different degradation times (2-12 days) for a given fabric construction at the same pressure. This indicates that the 2-day exposure to fluid was adequate to affect the transverse properties of the yarns.

4.4.3. Elastic Recovery

An artery is subjected to a frequency of 1 Hz or greater on a continuing basis. Thus, for long term success of a graft, the latter should continue to be compliant from cycle to cycle, i.e. it should be highly elastic at the loads it is subjected to. A recovery value of 1 or 100% indicates the recovery is complete and the material is perfectly elastic. On application of stress or pressure by a fluid flowing through the tube, the latter undergoes deformation in radial direction. Complete recovery of the diameter is important to maintain uniform flow of a fluid through the tube.

4.4.3.1. Effect of number of cycles on elastic recovery

Elastic recovery properties were assessed using a cyclic test that involved 5 cycles of loading and unloading. Figure 4.11. shows the profile of this behavior. A specimen was extended to a load limit of 50gf and then unloaded in order for the elongation to recover. This magnitude of load limit was chosen to offer a reasonable amount of load-elongation data so that the behavior corresponding the desired pressures (100 mmHg and 200 mmHg) could be

determined. While unloading, the specimen does not usually follow the same path and some amount of energy is lost. This phenomenon is called ‘hysteresis’. Also, some amount of elongation is not recovered which is due to permanent set in the structure.

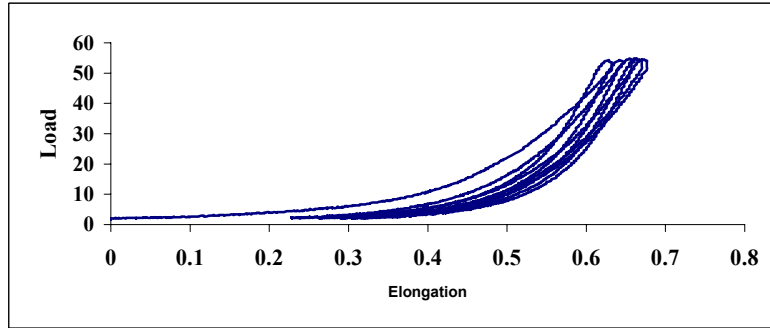


Figure 4. 11. Load-Elongation Behavior under Cyclic Test

In this work, the loading and unloading was repeated five times, after which it was observed that the results fell on a single loop. At this stage, the specimen is considered mechanically conditioned and became almost perfectly elastic. This means that the elongation in the specimen was now almost completely recovered. This is confirmed for the results given in Figure 4.12.

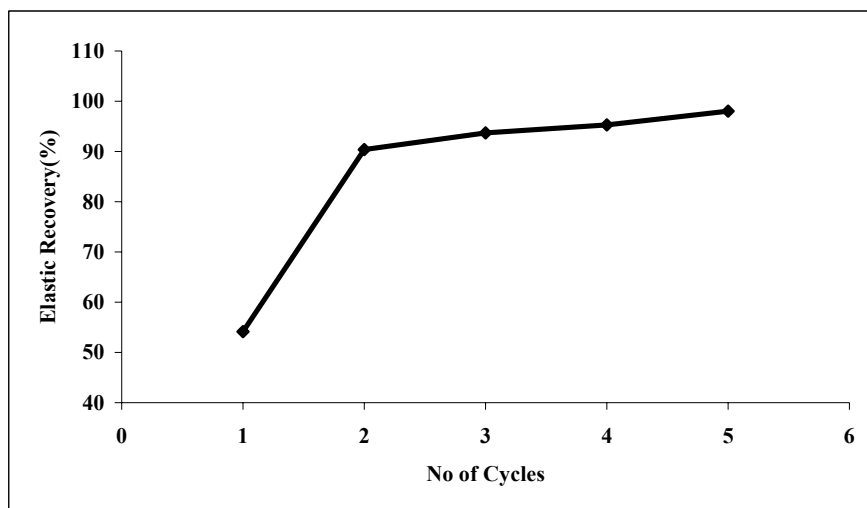


Figure 4. 12. Effect of Number of Cycles on Elastic Recovery

It is seen that the elongation was greatly recovered after second cycle and it was almost completely recovered after the fourth or the fifth cycle. This trend was found to be present in all samples of this research. Individual values of elastic recovery for cycles 1, 2 and 5 are listed in Table 4.12.

The statistical tests conducted on the mean values showed that the difference in the elastic recovery between cycle 1 and 2 were significant at 95 % confidence level and the ANOVA table are given in appendix E.

Table 4. 12.Elastic Recovery values after cycles 1, 2 and 5 (%)

Sample (no. of ends/ pick density)	1st	2nd	5th
Undegraded			
50/44	62.37±5.90	91.78±3.21	100.02±3.44
50/32	60.40±8.86	93.49±5.50	97.77±0.77
44/38	61.61±9.41	92.67±6.45	95.76±2.47
38/44	67.96±3.96	90.91±1.06	97.46±0.55
38/32	65.72±7.79	94.14±3.64	96.12±2.81
2 Days Degraded			
50/44	51.56±5.17	89.06±6.39	96.95±1.76
50/32	50.26±11.77	92.06±2.43	98.58±1.67
44/38	58.63±4.90	89.90±2.67	99.04±2.27
38/44	61.43±5.26	96.57±5.73	97.84±3.32
38/32	62.99±7.31	93.40±3.57	99.40±3.78
4 Days Degraded			
50/44	55.21±6.29	90.14±2.30	96.65±1.88
50/32	57.94 ±5.21	92.97 ±1.61	98.55 ±2.16
44/38	51.41±8.53	89.97±2.92	96.57±3.32
38/44	65.33±2.76	89.78±3.81	98.15±4.4
38/32	93.47±6.52	99.35±2.60	98.47 ±6.06
6 Days Degraded			
50/44	56.51±5.54	88.89±2.30	96.08±1.89
50/32	59.94 ±4.53	91.24 ±3.61	95.35 ±4.16
44/38	58.38±4.62	87.85±1.66	96.00±1.64
38/44	62.32±5.62	91.07±4.31	97.50±7.86
38/32	58.57±5.86	91.30±5.78	94.78±2.49

Sample (no. of ends/ pick density)	1 st	2 nd	5 th
8 Days Degraded			
50/44	53.79±6.87	88.28±3.09	97.06±3.4
50/32	56.74 ±3.85	90.97 ±3.56	96.55 ±3.78
44/38	53.94±3.35	85.92±1.54	96.88±1.1
38/44	64.40±2.56	90.68±2.55	97.15±3.91
38/32	62.11±5.92	89.85±2.14	96.86±2.29
10 Days Degraded			
50/44*	52.25*	86.77*	95.35*
50/32	53.94 ±6.85	88.97 ±1.61	97.55 ±2.16
44/38	54.35±4.10	87.80±3.24	96.86±1.92
38/44	57.02±8.20	84.29±8.11	97.59±2.15
38/32	60.09±6.50	85.55±2.82	96.32±3.16
12 Days Degraded			
50/44	49.47±3.72	85.16±0.56	95.10±2.26
50/32	51.65±2.61	85.96±1.93	93.59±1.88
44/38	62.35 ±1.81	94.47 ±4.43	97.36 ±1.67
38/44	54.67±3.40	88.54±1.95	96.57±3.09
38/32	56.14±4.34	89.18±1.96	95.87±2.03

The mean values are averages of 5 specimens. Given after the mean value is the value of 1 standard deviation

4.4.3.2. Effect of Degradation Time and Graft Construction on Elastic Recovery

The trends illustrated in Figures 4.13 and 4.14 indicate that elastic recovery in general increased with increase in pick density. This effect was noted for the values of elastic recovery after 1st cycle of loading and unloading. The effect was less prominent after first two cycles because the material became mechanically conditioned at that point (Figure 4.14). Amount of load sustained by a single yarn is less when pick density is increased, as same load is distributed over more number of threads. This showed cause less extension in the yarn. In visco-elastic materials, usually, the lower the extension, the greater the possibility that it is in elastic region. Accordingly, elastic recovery noted is greater with greater number of picks.

On the effect of the number of ends, it is noted that elastic recovery after 1st cycle is less with larger number of warps. This is probably due to the greater number of cross over points with more warps causing greater resistance to recover.

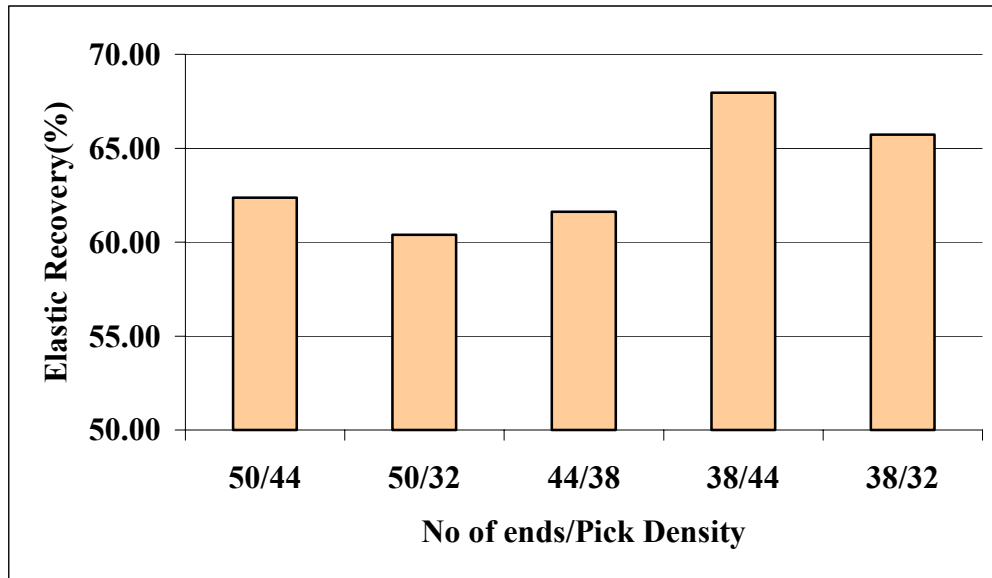


Figure 4. 13 Effect of Pick Density on Elastic Recovery after 1st cycle (Undegraded)

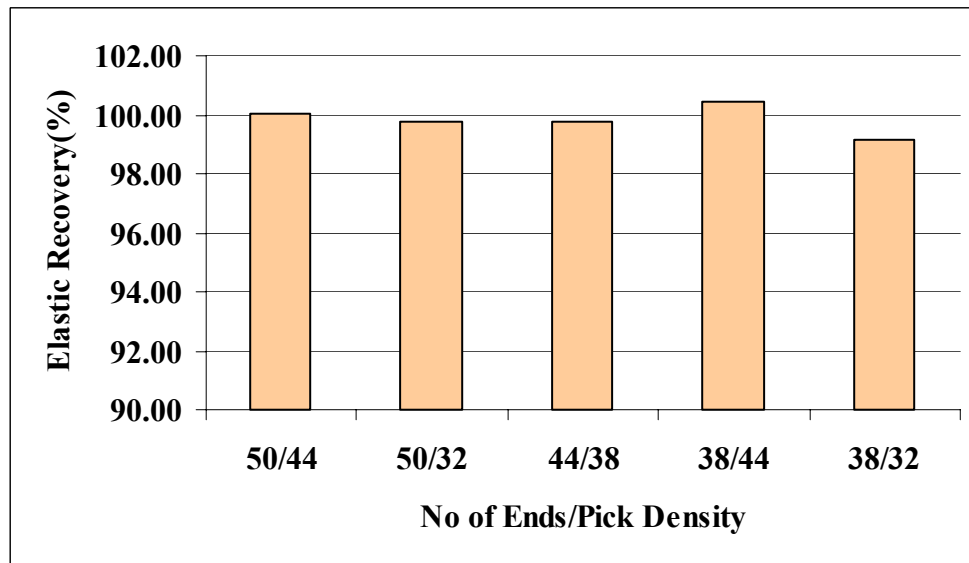


Figure 4. 14 Effect of Pick Density on Elastic Recovery after 5th Cycle (Undegraded)

The general trends illustrated in Figure 4.14 indicate that elastic recovery in general decreased with increased degradation time. This effect was mostly prominent and significant for the values of elastic recovery after 1st cycle of loading and unloading. A decrease in elastic recovery with degradation is expected to be due to the breakages of cross-links between chains. These cause the chain to slip past each other and under go plastic deformation. Also in general the elastic recovery increased with decrease in number of ends.

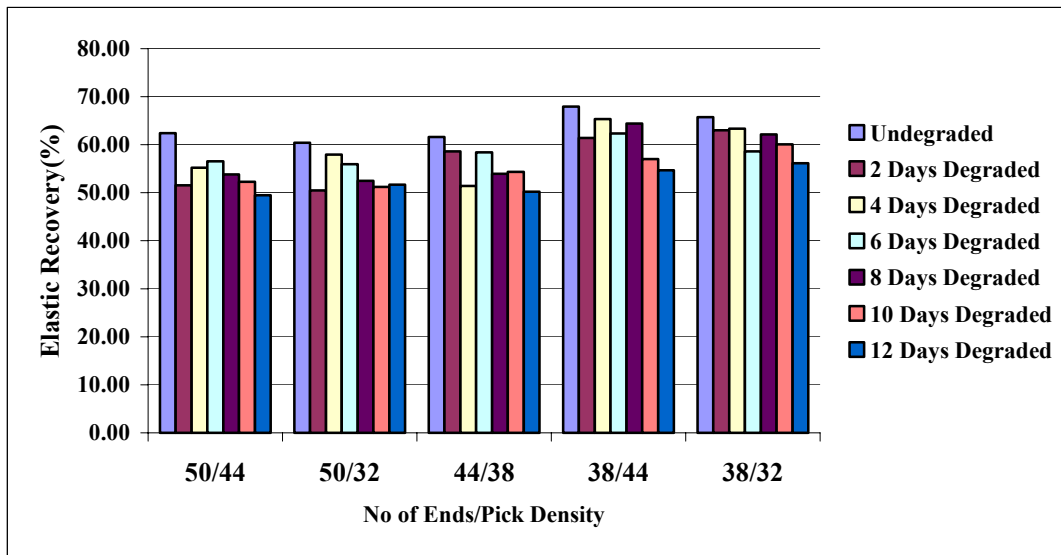


Figure 4. 15 Effect of Degradation Time on Elastic Recovery after 1st cycle

4.4.4. Compliance of The Woven Grafts

Mismatch of compliance between host and prosthetic arteries has been considered to be the major problem in the success of small diameter grafts in bypass surgeries. Compliance is characterized by the ratio of strain to stress at a given stress value. In our tests, compliance was determined by measuring the amount of strain a tube produced, at a given value of applied stress and taking the ratio of the two.

In the transverse direction, the weft yarn is expected to be the main component that contributes towards the mechanical properties of the tubes. The warp yarns perhaps also play some role, as they apply some resistance against the elongation of weft yarns due to friction at crossover points.

As seen in section 4.1.2., degradation time has a significant effect on the mechanical properties of the PLGA yarn. Therefore it is believed that degradation time would also have an effect on the compliance of the tubular structures. The results related to compliance are given in table 4.12.

4.4.4.1. Effect of Pressure on Compliance of Tubular Structures

Typical load-elongation behavior of a tubular fabric stretched in transverse direction is shown in Figure 4.16. Initially, as the specimen is elongated, the load developed in the specimen is low. The rate at which the load builds up increases as the elongation increases. The initial behavior can be ascribed to the presence of ‘crimp’ in the fabric due to interlacement of yarns. The load starts to develop in the fabric as the yarns in the fabric straighten out. The pressure acting on a fabric of given area is directly proportional to the load developed. As the slope of the graph increases with increase in elongation, the compliance, which is the ratio of strain to stress (or elongation to load), should decrease with increase in elongation. Therefore, the compliance values are lower at 200-mmHg pressure

than those at 100-mmHg pressure. This is observed from the data in Table 4.13 and is more clearly seen in Figure 4.17.

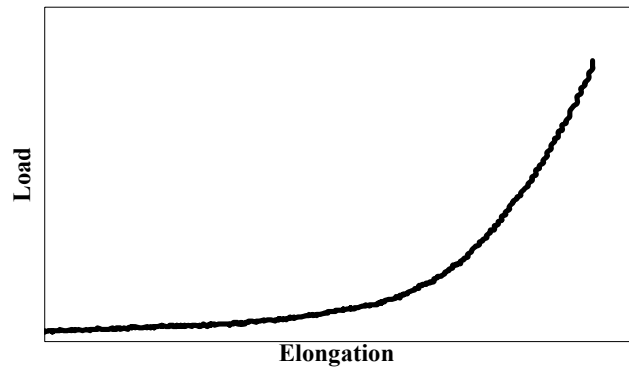


Figure 4. 16 Load-Elongation Behavior of Tubular fabric

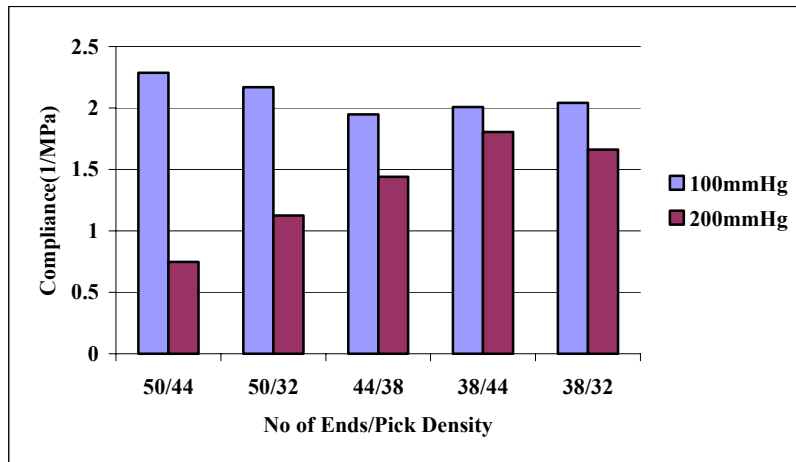


Figure 4. 17 Effect of Applied Pressure on Compliance (Undegraded)

Table 4. 13 Compliance Values of Tubular Structures (1/ MPa)

Sample (no. of ends/ pick density)	Compliance (1/Mpa)	
	100mmHg	200mmHg
Undegraded		
50/44	2.29±0.27	0.75±0.22
50/32	2.17±0.15	1.13±0.16
44/38	1.95±0.10	1.44±0.06
38/44	2.01±0.33	1.80±0.31
38/32	2.04±0.15	1.66±0.13
2 days		
50/44	2.52±0.26	2.13±0.16
50/32	2.43±0.22	1.50±0.20
44/38	2.85±0.10	2.64±0.08
38/44	2.35±0.22	1.88±0.13
38/32	2.78±0.29	1.97±0.17
4 days		
50/44	2.65±0.14	2.49±0.17
50/32	2.56±0.22	2.16±0.18
44/38	2.65±0.39	2.05±0.28
38/44	2.65±0.13	2.05±0.09
38/32	2.54±0.13	1.58±0.07
6 days		
50/44	2.43±0.42	1.80±0.29
50/32	2.35±0.24	1.26±0.11
44/38	2.54±0.21	1.71±0.28
38/44	2.57±0.32	1.55±0.28
38/32	2.60±0.18	1.61±0.15

Sample (no. of ends/ pick density)	Compliance (1/Mpa)	
	100mmHg	200mmHg
8 days		
50/44	2.52±0.30	2.02±0.44
50/32	2.63±0.19	2.23±0.15
44/38	2.77±0.32	1.40±0.20
38/44	2.50±0.42	2.32±0.27
38/32	2.65±0.12	1.56±0.13
10 days		
50/44	2.80±0.26	2.10±0.14
50/32	2.75±0.03	1.51±0.07
44/38	2.44*	1.26*
38/44	2.72±0.15	1.59±0.10
38/32	2.40±0.02	1.77±0.01
12 days		
50/44	2.86±0.22	1.91±0.15
50/32	2.51±0.06	1.49±0.06
44/38	2.77±0.28	2.40±0.21
38/44	2.18*	1.95*
38/32	2.38±0.12	1.64±0.06

The mean values are averages of 5 specimens. Given after the mean value is the value of 1 standard deviation

* Based on One specimen

4.4.4.2. Effect of Degradation Time on Compliance of Tubular Structures

The effect of degradation time on compliance is shown in Figures 4.18 and 4.19. Degradation time affects compliance as expected. As mentioned earlier, degradation time has significant effect on yarn mechanical properties. The modulus of the yarn decreased with increase in degradation time (Table 4.3 and Figure 4.20). Since compliance is reciprocal of modulus, it is expected that compliance will increase with degradation time. The results have substantial scatter but in general one can conclude that the trend is in agreement with the

expected result, most particularly at 100 mmHg. The effect is consistent and profound when one compares the compliance of the 2 day degraded material with that of the undegraded material. This is evaluated more in detail later. The scatter in results is expected to be due to the unpredictable effect degradation has on the frictional properties at cross over points.

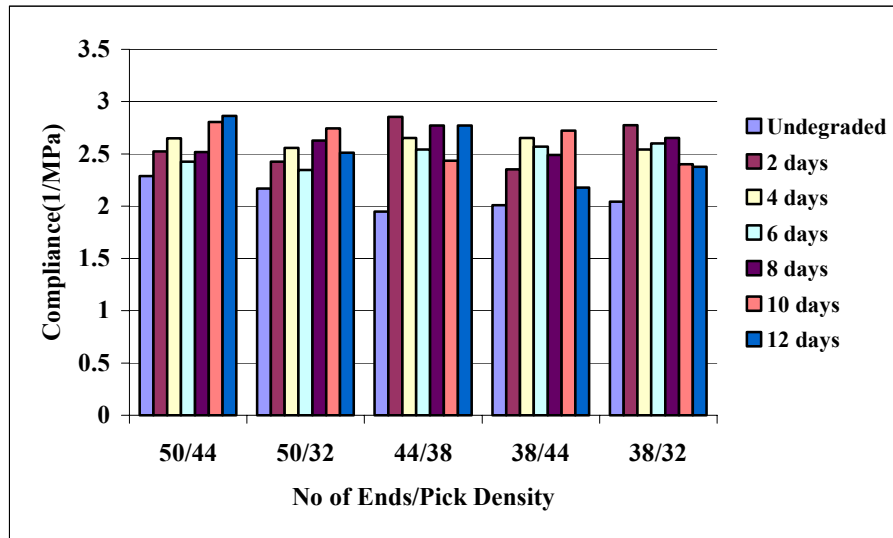


Figure 4. 18 Effect of Degradation Time on Compliance (100 mmHg)

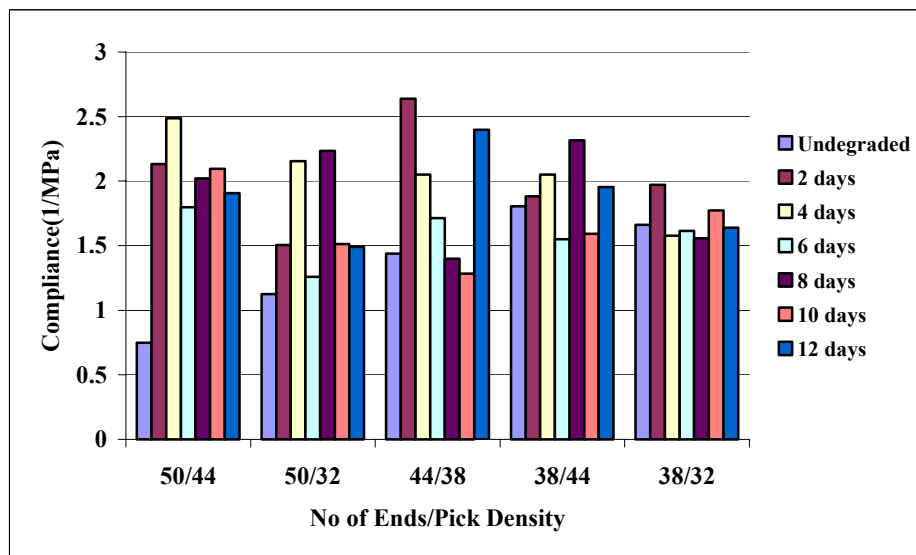


Figure 4. 19 Effect of Degradation Time on Compliance (200 mmHg)

Figure 4.21 shows a comparison between compliance of undegraded tubular fabrics and 2 days degraded tubular fabrics. From the studies done on yarn, it is known that the modulus decreased by more than 50 percent within the first 48 hours of degradation time (Figure 4.20.). It follows that compliance should be higher after degradation. The comparison is shown in figure 4.21 and 4.22. The statistical analysis indicated that, the difference between the compliance of the undegraded tubular structures and the two day degraded structures was significant at 95% confidence limit. After 2 days, the behavior becomes irregular not only because degradation affected the properties variously at crossover points but also the change in the mechanical properties of the yarn themselves was not uniform (Figure 4.19). The ANOVA table is given in appendix E

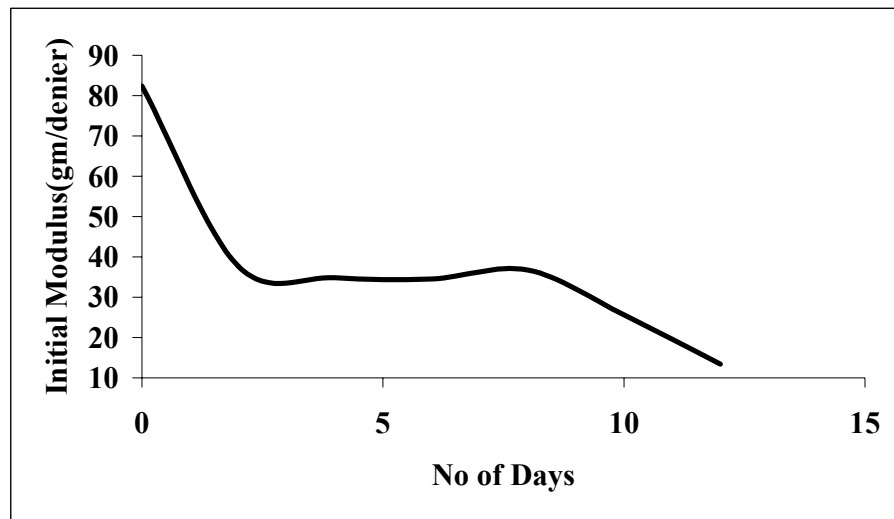


Figure 4. 20 Modulus of 56 Denier PLGA Yarn after Hydrolytic Degradation

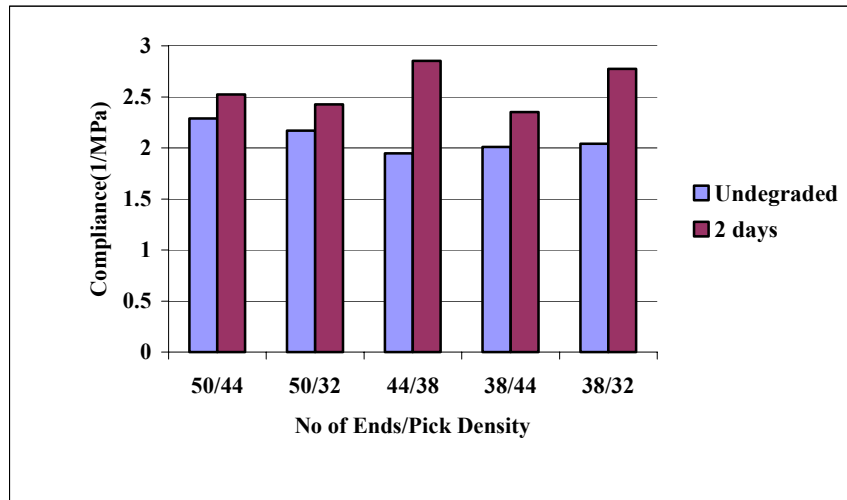


Figure 4. 21 Effect of Degradation on Compliance (100 mmHg)

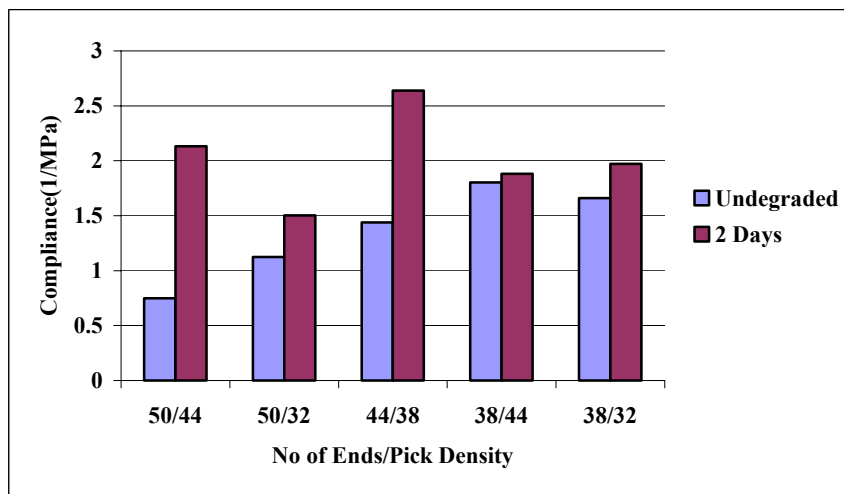


Figure 4. 22 Effect of Degradation on Compliance (200mmHg)

Structure to structure difference are present in the results above (figure 4.17, 4.21, 4.22) but an in depth analysis would be needed to model the effect and understand the exact causes of these. Such analysis, critical for the design of grafts, would be the subject of follow up study.

4.5 Porosity

Another objective in this research has been to identify the factors that influenced porosity of the woven tubes and examine the nature of their effects. Of greater interest was the change in the porosity of the tubes subjected to hydrolytic degradation.

The number of warp yarns and pick density used were two of the primary factors that could affect the porosity; they showed a direct effect on the porosity value. As seen in Figure 4.23, higher pick density gave lower porosity and vice versa. This is because with more pick density, there is less spacing between the adjacent picks. This leads to a fabric with greater cover and smaller voids.

In Section 4.2.2, we saw that the diameter of graft did not increase linearly with increase in number of ends. It rather followed an exponential trend. Due to this non-linear behavior in diameter increase, the end density which is given by the number of ends per inch was lower in tubes with larger number of ends or larger diameter. Thus higher number of ends gave lower end density and ultimately higher porosity in the graft and vice versa. Table 4.14, gives a summary of the porosity values obtained.

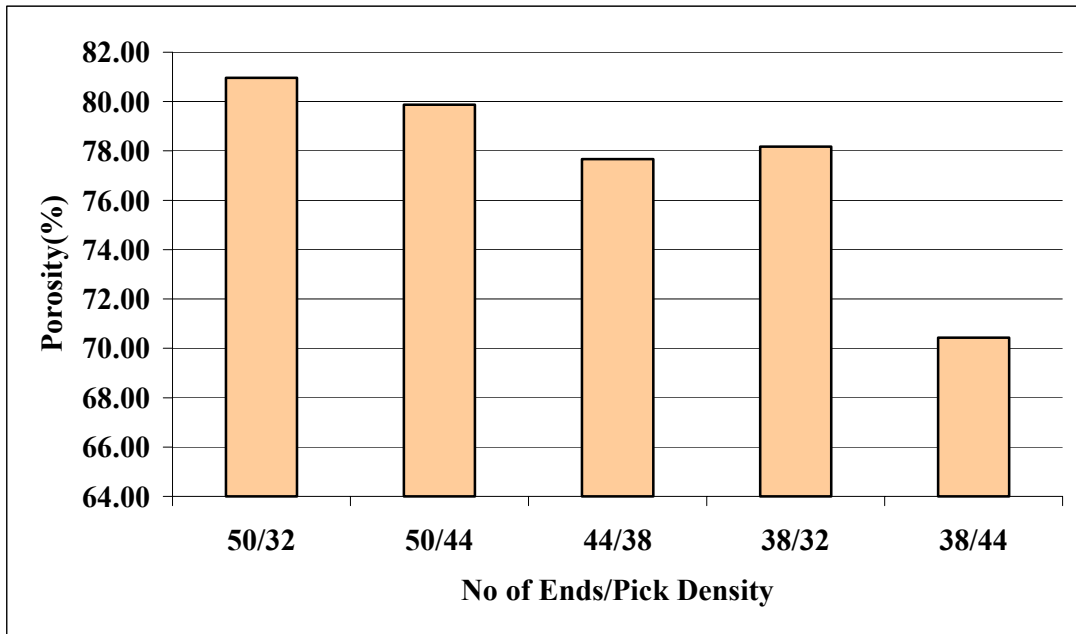


Figure 4. 23 Effect of No. of Ends/Pick Density on Porosity of Undegraded Structures

Table 4. 14 Tubular fabric Porosity Values for Different Degradation Time

Sample (no. of ends/ pick density)	Porosity (%)
Undegraded	
50/32	80.97
50/44	79.87
44/38	77.67
38/32	78.18
38/44	70.43
7 Days Degraded	
50/32	80.31
50/44	80.27
44/38	77.02
38/32	77.73
38/44	70.34

Sample (no. of ends/ pick density)	Porosity (%)
14 Days Degraded	
50/32	79.63
50/44	79.55
44/38	78.03
38/32	78.48
38/44	70.74

The mean values are averages of 5 specimens. Given after the mean values is the value of 1 standard division.

* Based on one specimen.

4.5.1 Effect of Degradation Time on Porosity

In figure 4.24, the porosity values are illustrated as a function of degradation time for different structures. The porosity did not change significantly until 14 days of degradation. The mass loss during this period was less than 11%. Although there was a mass loss, the diameter also did not change during this period of degradation (section 4.2) indicating that macroscopic porosity of the grafts was maintained during this period. One could expect that the microscopic porosity started to increase with degradation after first week.

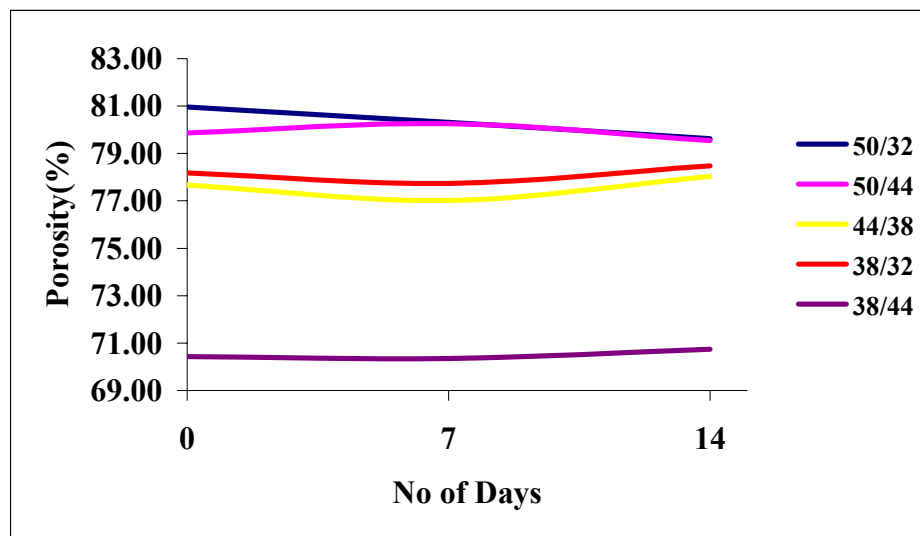


Figure 4. 24 Effect of Degradation Time on Porosity of Grafts

5. SUMMARY AND CONCLUSION

Previous studies have reported that totally resorbable prostheses woven from biodegradable polymers such as PLGA and implanted into animal models elicited healing reactions, which established regenerated tissue conduits. However the present literature lacks information related to manufacturing of such prostheses. Also there are very few publications, which describe the mechanical behaviors of these materials when used in the woven form. Thus, this research focused on determining the design and manufacturing conditions of biodegradable woven small diameter vascular grafts and understanding their behavior was undertaken with following objects in mind. First, to determine the process parameters that are required to make tubular structure using PLGA yarns. Second, to determine the various heat setting conditions required to convert these woven structures, which are flat initially, into circular tubes. Third, to determine the mechanical performance of such tubes in terms of elastic recovery and compliance after subjecting them to degradation in hydrolytic solutions. Fourth it was necessary determine the changes in porosity occurring over time as these materials are subjected to degradation environment.

Degradation studies performed on the yarns demonstrated that the mechanical properties changed considerably with the degradation time. It was observed that loss of tensile strength was rapid and that load-bearing ability reduced to zero within two weeks. It was also noted that in comparison to the strength, the loss mass loss lagged behind, indicating that bulk erosion took place in the polymer. There was no significant mass loss detected during the first week. The mass loss was a 11% at the end of two weeks and 50% at

the end of four weeks. The mass loss data fitted to an exponential model yielded a correlation coefficient, R^2 , of 0.97.

In order to determine the optimum conditions for heatsetting the flat woven structures, heat setting studies were performed on the yarns. These studies demonstrated that the mechanical properties of the yarns changed considerably with the conditions used. 150°C temperature and 60-second dwell time of heat setting gave optimum mechanical properties and hence were used to heat set the woven tubular structures.

Degradation studies performed on tubular fabrics demonstrated that mass loss followed an exponential trend, which was similar to the trend found on the yarns. The number of ends and the pick density did not have an effect on mass loss, which showed that the latter was only a characteristic of the polymer material.

The results obtained from the testing of grafts for compliance and elastic recovery showed that the degradation time and structural parameters effected these properties. It was observed that the elastic recovery of the structure largely depended upon the number of loading and unloading cycles to which the structure was subjected. Elastic recovery in the first cycle was the lowest, as expected. The general trend indicated that elastic recovery in general decreased with an increase in degradation time. This effect was prominent for the values of elastic recovery in the 1st cycle of loading and unloading. Also, in general, the elastic recovery increased with decrease in number of ends.

Elastic recovery in the second cycle was excellent. In the fifth cycle, all samples of this research showed a recovery of more than 95% after stretching irrespective of construction parameters.

Degradation time had significant effect on the mechanical properties of both the constituent yarn and the graft. The modulus decreased with increase in degradation. Since compliance is the reciprocal of modulus, it increased with degradation time. With some exceptions the general trends were in agreement with expected result. The statistical analysis also showed that, there was a significant difference between the compliance of the undegraded tubular structures and the two-day degraded structures.

Porosity of the tubular structures was dependent on the number of warp yarns and pick density used. The porosity increased with decrease in pick density and end density and vice versa. Since yarn diameter did not change during 2 weeks of degradation, macroscopic porosity of the grafts was maintained during this period. Although the mass did not change during the first week, it decreased by about 11 % during the second week. Accordingly one could expect that the microscopic porosity started to increase with degradation time after the first week.

6. FUTURE RECOMMENDATIONS

Although this work has given some insight on the behavior of woven small diameter grafts constructed using a biodegradable material, further investigation remains necessary in the following areas:

- Understand and model the effect of graft construction parameters on compliance.
- Evaluate the effect of degradation on the overall porosity (macroscopic and microscopic) and pore size distribution of the biodegradable prostheses.
- In bypass applications, a prosthesis should last longer than is possible with a 100 % degradable material. It is proposed that a degradable yarn be combined with a nondegradable yarn in variety of ways and the grafts constructed from these be examined for their degradation and mechanical properties and for the potential they present in clinical applications.

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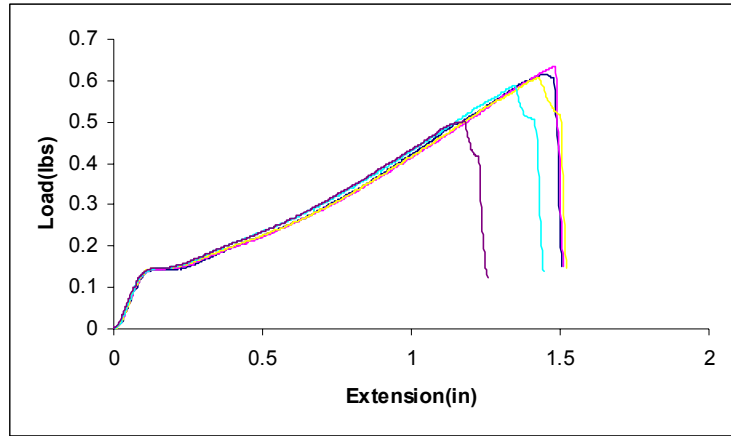
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APPENDICES

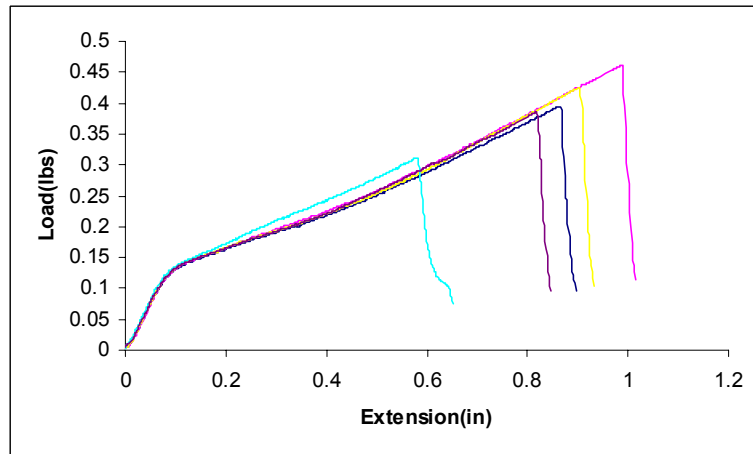
Appendix A: Load Elongation Curves

Given in this appendix are the load-elongation curves of undegraded and degraded yarns

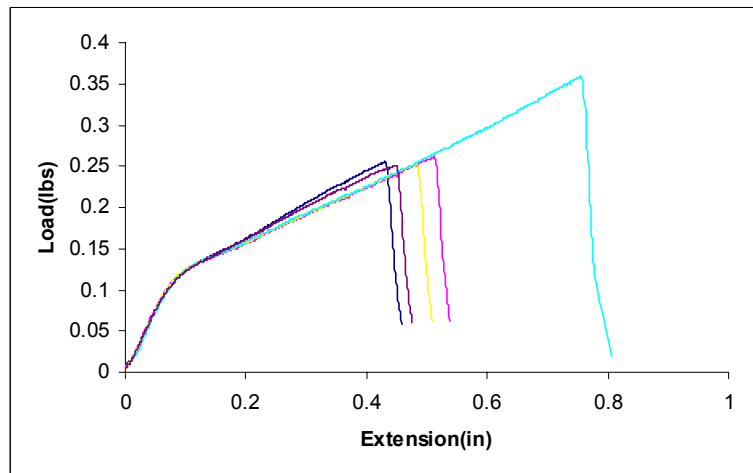
Undegraded



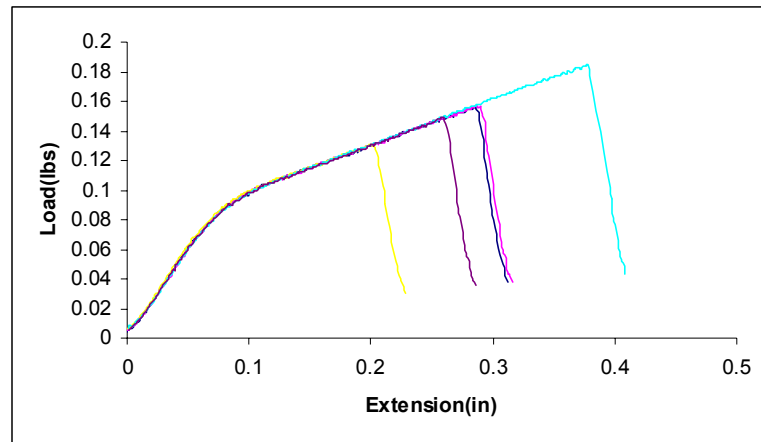
2 Days Degraded



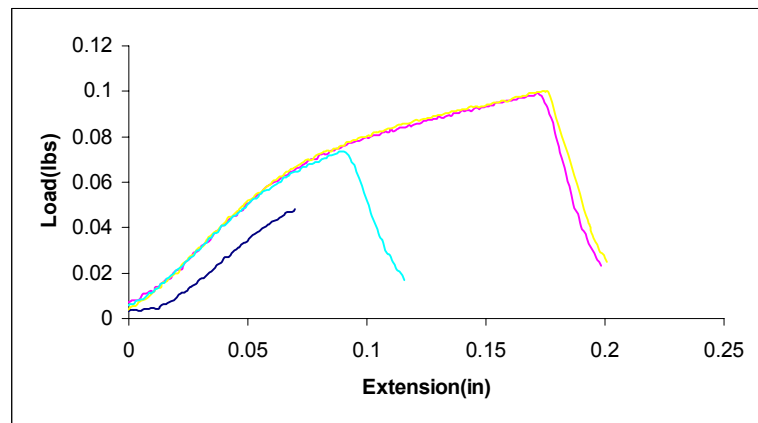
4 Days Degraded



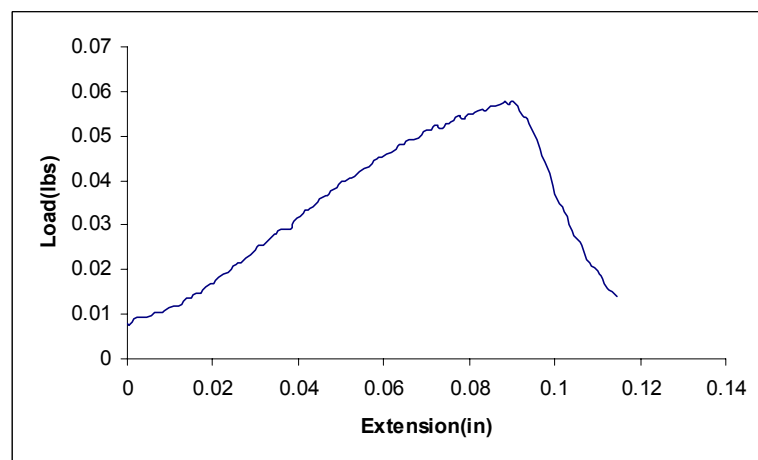
6 Days Degraded



8 Days Degraded



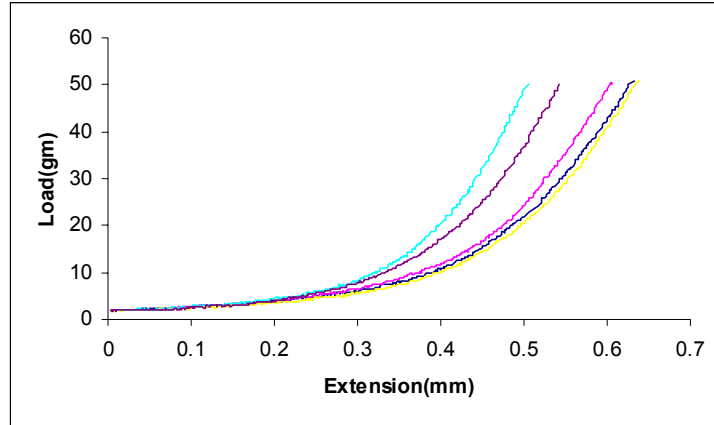
10 Days Degraded



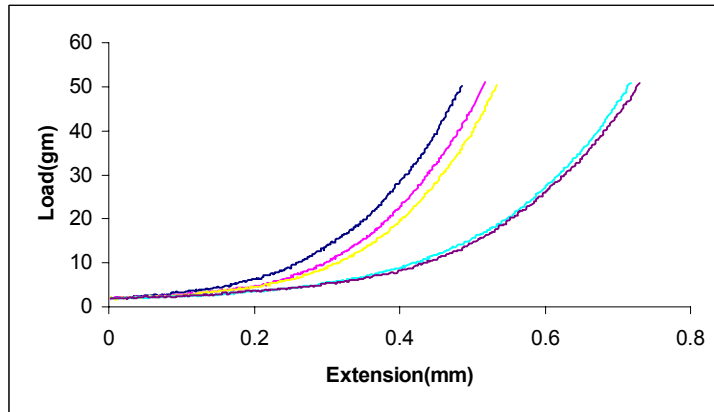
Appendix B: Load Elongation Curves

Given in this appendix are the load-elongation curves of all undegraded and degraded tubular structures

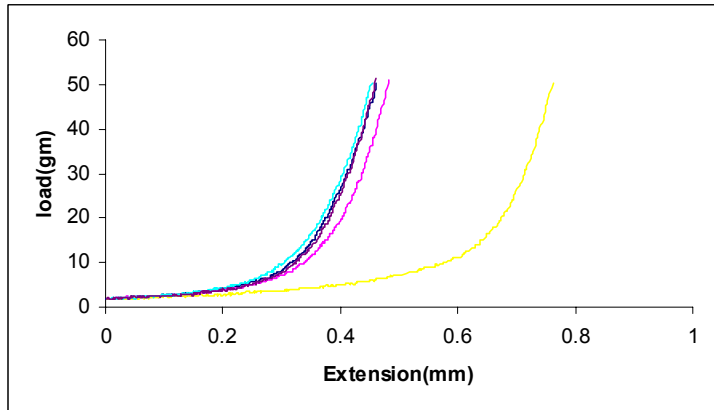
Undegraded-38/32



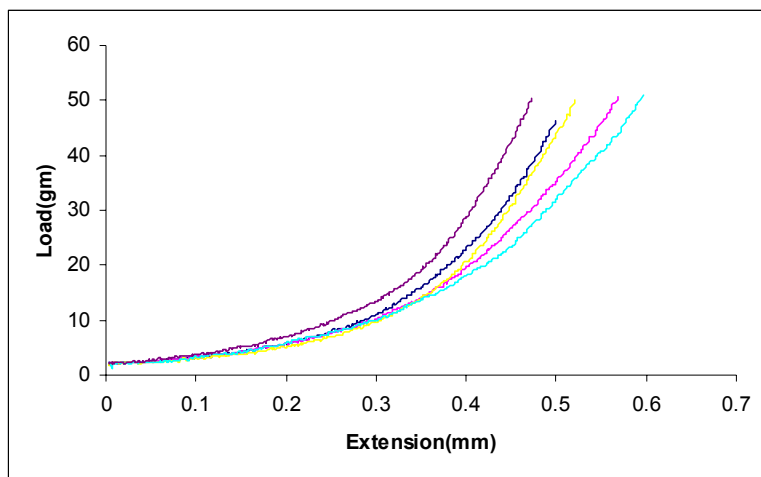
Undegraded-38/44



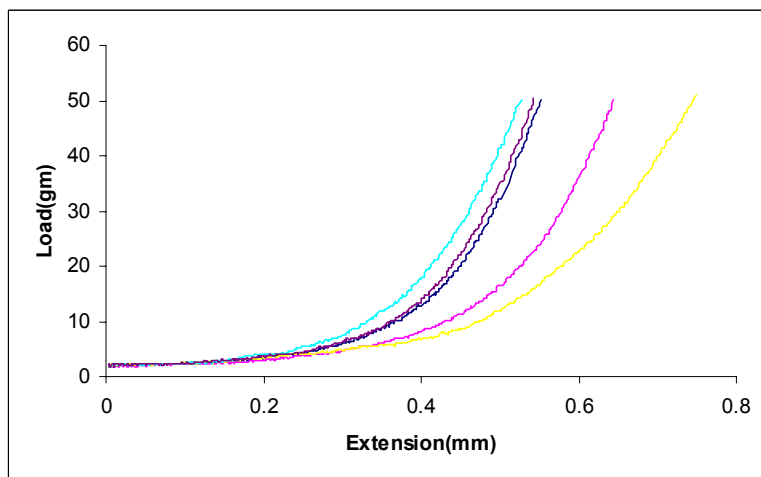
Undegraded-44/38



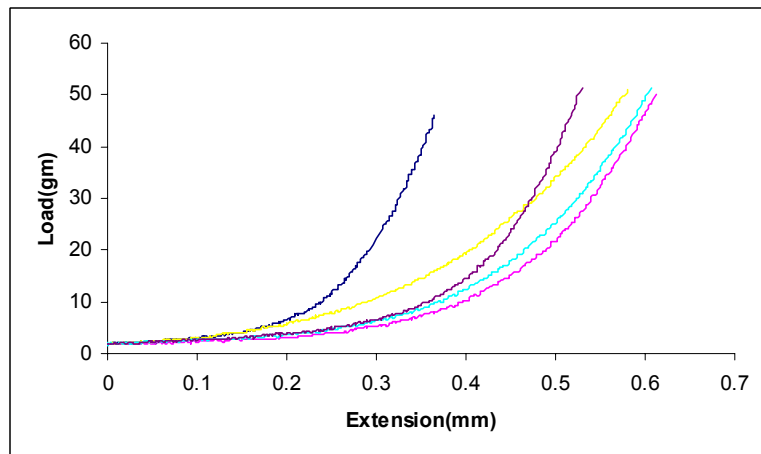
Undegraded-50/32



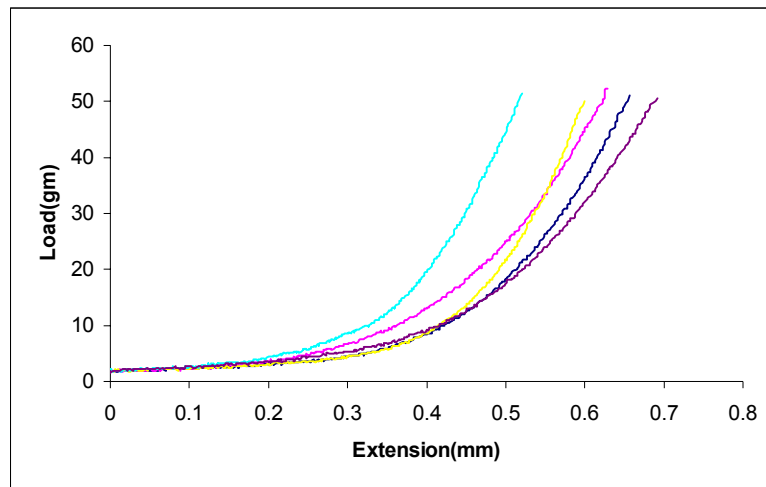
Undegraded-50/4



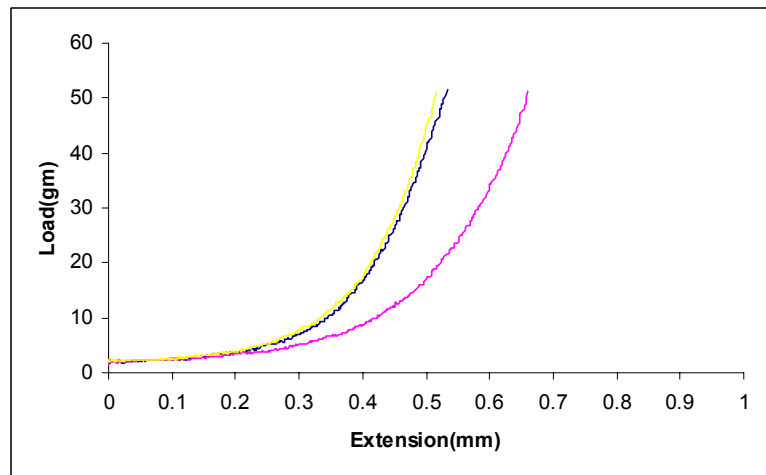
2 Days-38/32



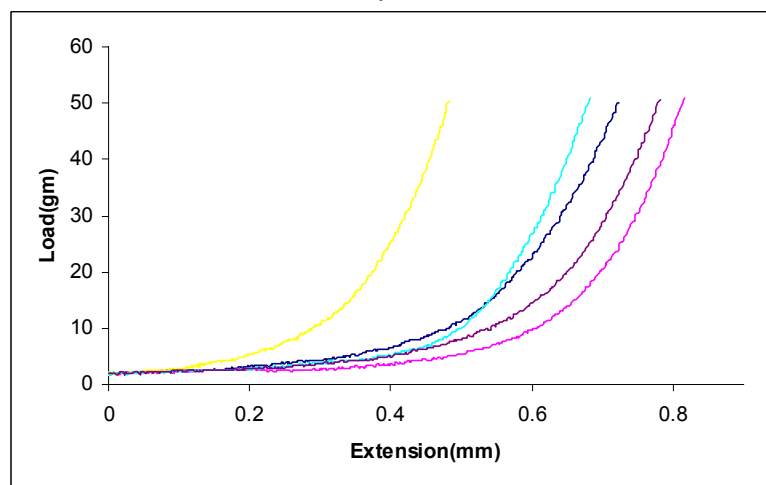
2 Days-38/44



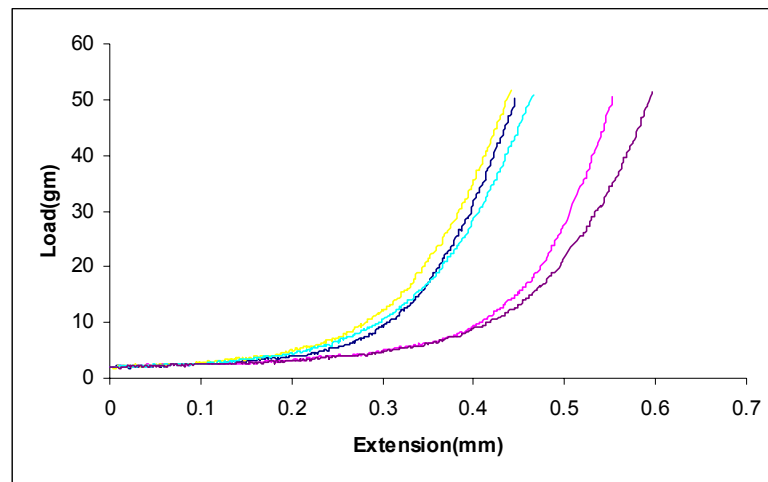
2 Days-50/32



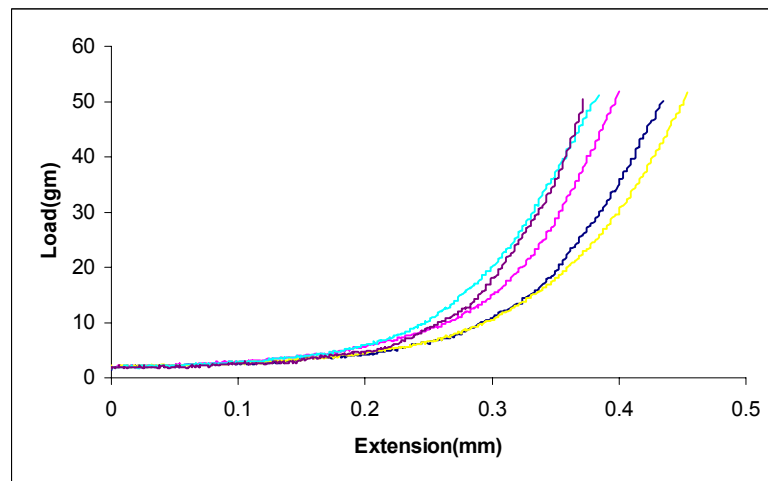
2 Days-50/44



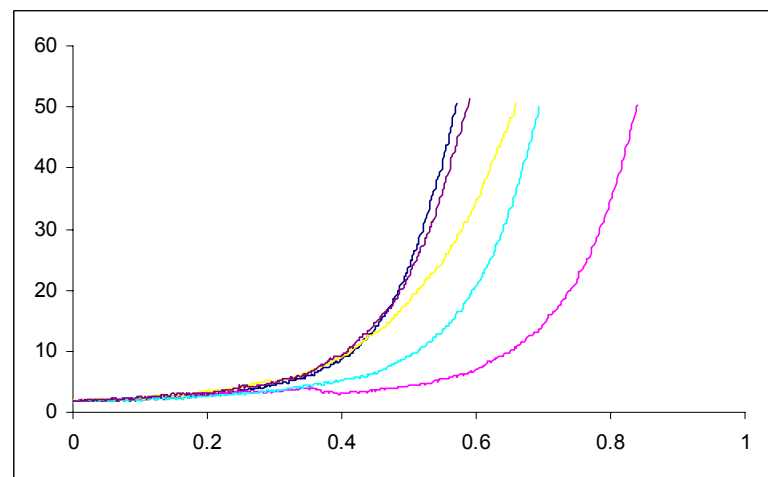
4 Days-38/32



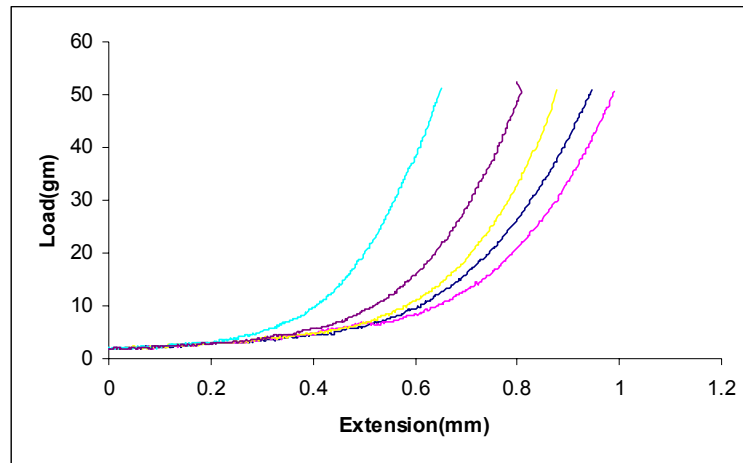
4 Days-38/44



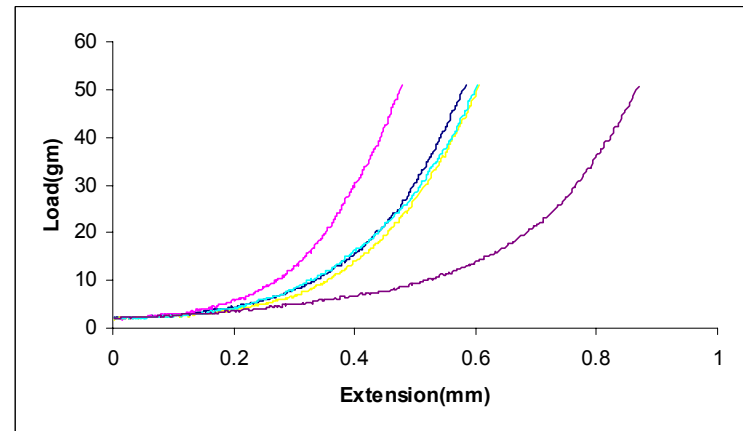
4 Days-44/38



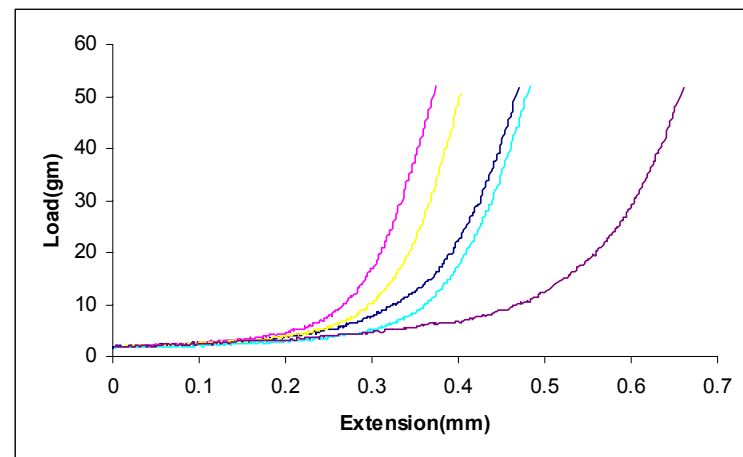
4 Days-50-44



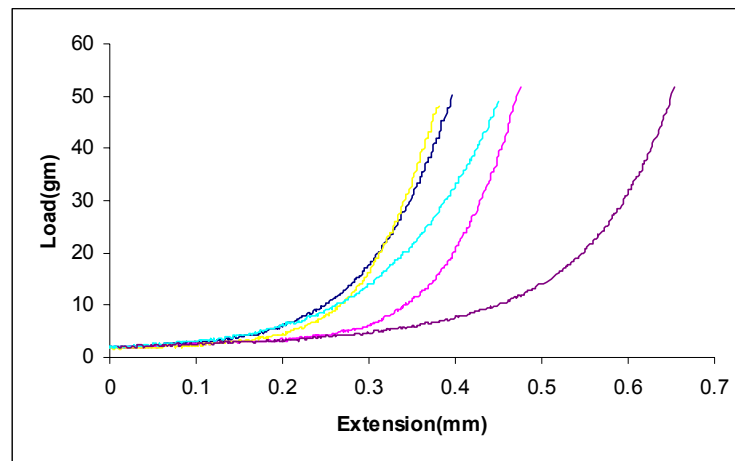
6 Days-38-32



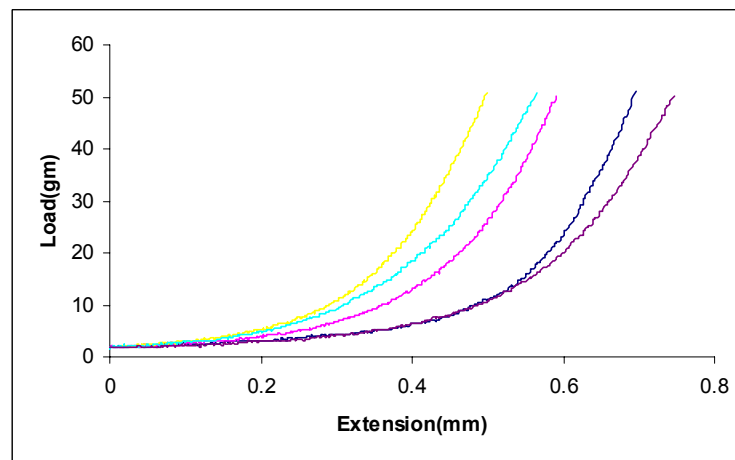
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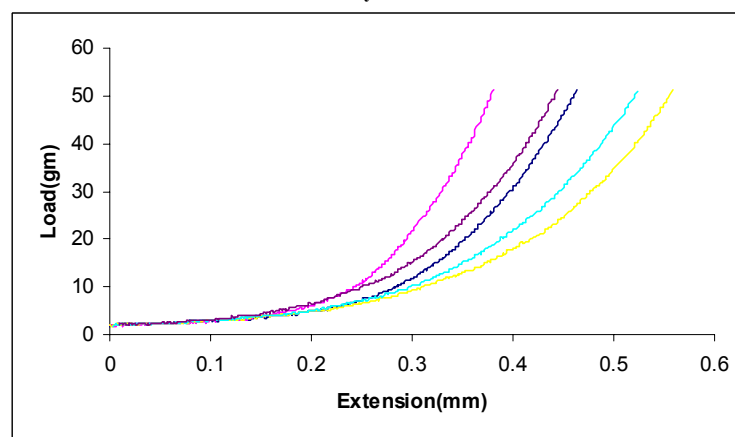
6 Days-44-38



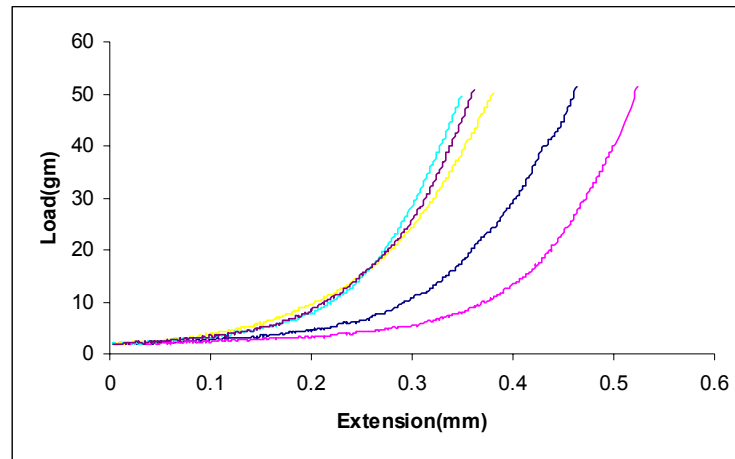
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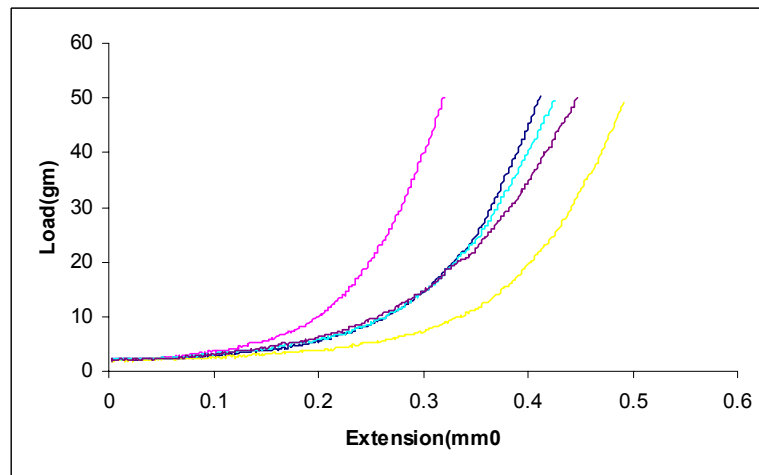
8 Days-38-32



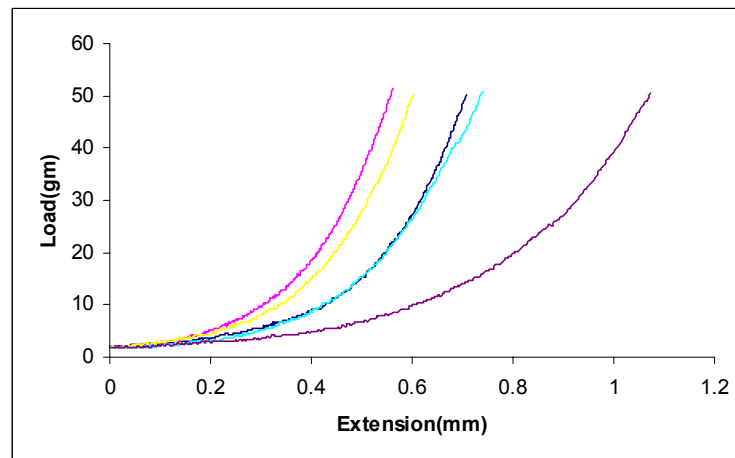
8 Days-38-44



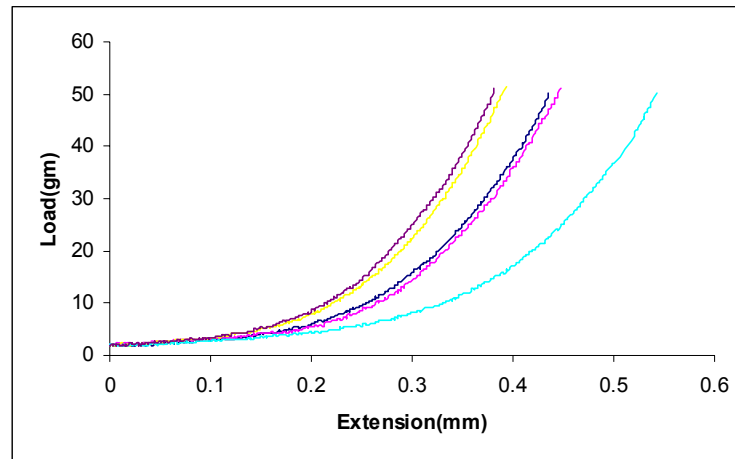
8 Days-44-38



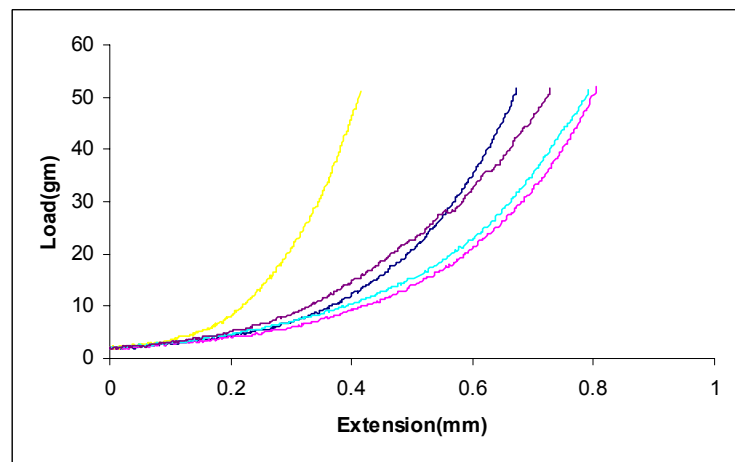
8 Days-50-44



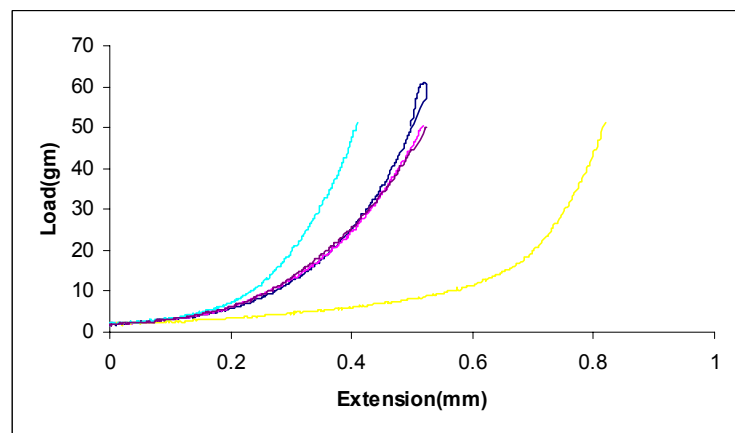
10 Days-38-32



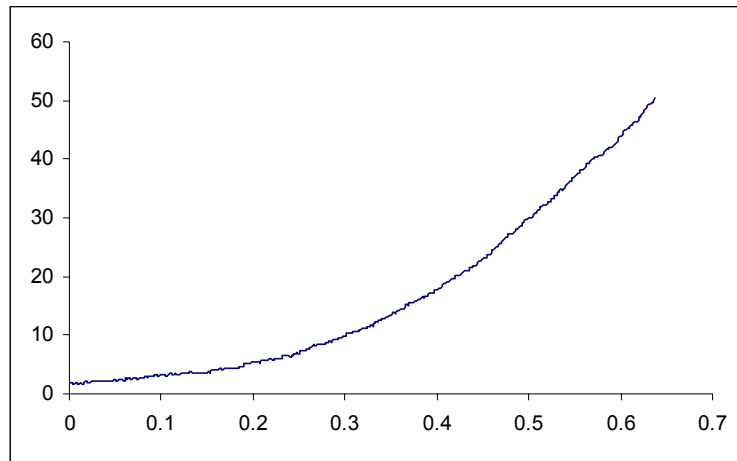
10 Days-38-44



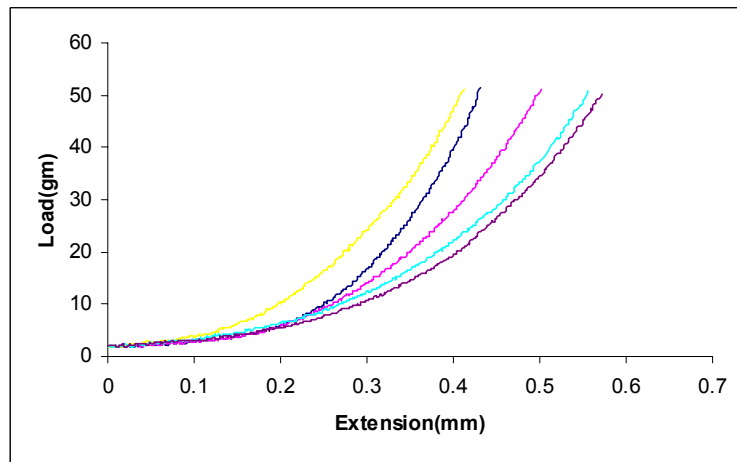
10 Days-44-38



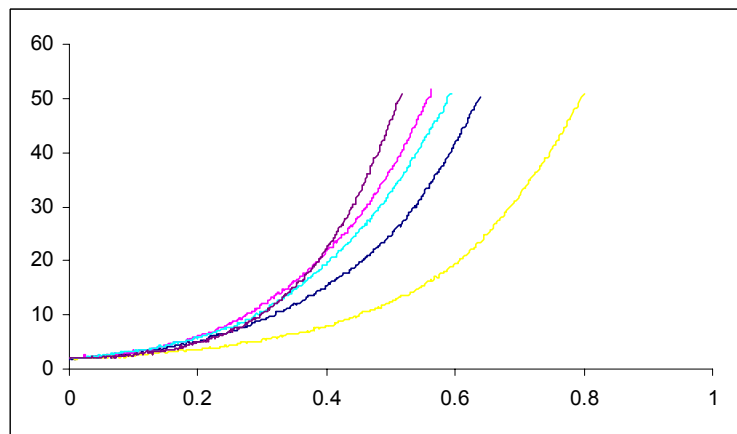
10 Days-50-44



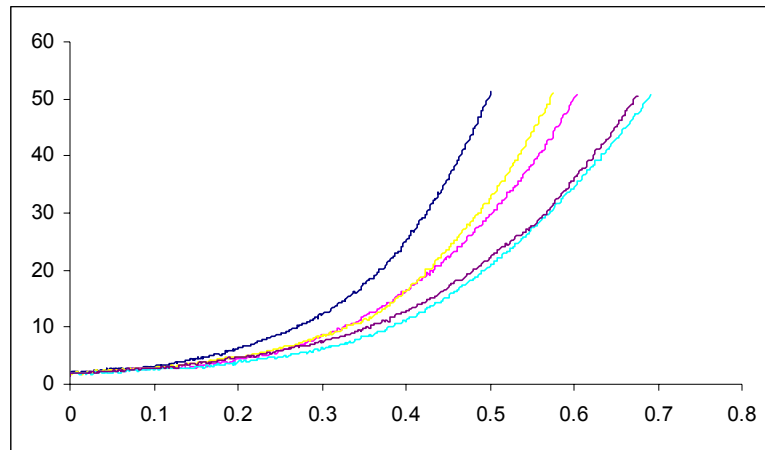
12 Days-38-32



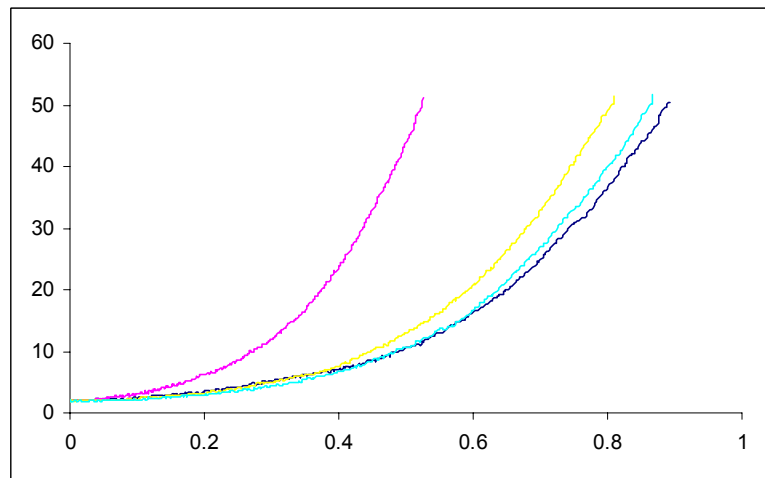
12 Days-38-44



12 Days-50-32



12 Days-50-44



Appendix C: Compliance Calculation

$$\text{Compliance} = \frac{\text{Strain at given Stress or Pressure}}{\text{Stress or Pressure}}$$

$$\text{Load} = \text{Pressure} * \text{Area}$$

$$\text{Area} = 2 * \text{Width} * \text{Thickness}$$

$$1\text{mmHg} = 0.133\text{ KPa}$$

Example: (Undegraded-44/38)

Results:

Width (mm)	Thickness (mm)		Area Under stress (mm ²)	Pressure (mmHg)	Load (gf)	Corresponding Strain From Graph	Compliance (1/Mpa)
	At 100mmHg	At 200mmHg					
10	0.12	0.1	2.4	100	3.26	0.025	1.89
			2	200	5.43	0.037	1.40
10	0.12	0.1	2.4	100	3.26	0.027	2.06
			2	200	5.43	0.040	1.52
10	0.12	0.1	2.4	100	3.26	0.043	3.22
			2	200	5.43	0.065	4.55
10	0.12	0.1	2.4	100	3.26	0.025	1.85
			2	200	5.43	0.037	1.38
10	0.12	0.1	2.4	100	3.26	0.027	2.00
			2	200	5.43	0.039	1.46
Average				100	3.26	.029	1.95
				200	5.43	.044	1.44

Calculation:

For pressure 100mmHg:

$$\text{Area Under Stress (mm}^2\text{)} = 2 * 10 * 0.24 = 2.4$$

Load (gf), corresponding to the pressure of 100 mmHg, on the sample of area 1.8 mm²

$$= 13300 * (2.4 * 10^{-6}) / 0.0098 = 3.26\text{ gf}$$

Strain corresponding to the load of 3.26 gf is directly read from the load-elongation data of this sample and is = 0.25

$$\text{Compliance} = (0.025 * 1000) / 13.3 = 1.89$$

Appendix D: Thickness Testing

Thickness testing was carried out on a ‘SDL Thickness Gage’. Three specimens each of area 1.5 cm^2 were cut carefully from each sample and thickness measurement was carried out at four different available pressures. These pressures were 4.53, 11.31, 22.63 and, 45.26 KPa. The graph of Thickness against Pressure was plotted and regression equation was generated. Each table shows R^2 values for these equations. From these equations, thickness values corresponding to 100 mmHg and 200 mmHg were determined.

- Undegraded

Sample	Pressure KPa	Thickness (mm)				Model	R^2
		1	2	3	Average		
50/32	6.84	0.10	0.09	0.11	0.10	$y = 0.3039x^{-0.2301}$	$R^2 = 0.9832$
	17.10	0.07	0.08	0.09	0.08		
	34.21	0.06	0.07	0.08	0.07		
	68.41	0.05	0.06	0.07	0.06		
50/44	6.84	0.11	0.11	0.11	0.11	$y = 0.3173x - 0.1946$	$R^2 = 0.9973$
	17.10	0.10	0.09	0.09	0.09		
	34.21	0.09	0.08	0.08	0.08		
	68.41	0.08	0.07	0.07	0.07		
44/38	6.84	0.11	0.13	0.12	0.12	$y = 0.3431x - 0.1976$	$R^2 = 0.9973$
	17.10	0.09	0.11	0.10	0.10		
	34.21	0.08	0.09	0.09	0.09		
	68.41	0.07	0.09	0.07	0.08		
38/44	6.84	0.13	0.13	0.13	0.13	$y = 0.3482x - 0.1674$	$R^2 = 0.9961$
	17.10	0.11	0.11	0.12	0.11		
	34.21	0.09	0.10	0.10	0.10		
	68.41	0.09	0.09	0.09	0.09		
38/32	6.84	0.11	0.12	0.11	0.11	$y = -0.0414\ln(x) + 0.3089$	$R^2 = 0.9916$
	17.10	0.10	0.11	0.10	0.10		
	34.21	0.09	0.08	0.08	0.08		
	68.41	0.07	0.08	0.06	0.07		

- 2 Days Degraded

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.18	0.17	0.14	0.16	$y = -0.0615\ln(x) + 0.4026$	R2 = 0.9982
	9.19	0.14	0.14	0.12	0.13		
	18.38	0.12	0.11	0.10	0.11		
	36.77	0.10	0.09	0.09	0.09		
50/44	3.72	0.15	0.15	0.13	0.14	$y = 0.3531x - 0.1854$	R2 = 0.9849
	9.30	0.11	0.13	0.11	0.11		
	18.59	0.11	0.11	0.10	0.11		
	37.19	0.09	0.10	0.09	0.09		
44/38	5.62	0.13	0.13	0.14	0.13	$y = 0.3465x - 0.1566$	R2 = 0.9986
	14.04	0.12	0.11	0.12	0.12		
	28.08	0.10	0.10	0.11	0.10		
	56.17	0.09	0.09	0.10	0.09		
38/32	6.93	0.16	0.13	0.13	0.14	$y = 0.3933x - 0.1915$	R2 = 0.9963
	17.33	0.13	0.11	0.11	0.11		
	34.66	0.11	0.10	0.09	0.10		
	69.31	0.10	0.09	0.08	0.09		
38/44	5.78	0.13	0.12	0.12	0.12	$y = 0.3105x - 0.15$	R2 = 0.998
	14.44	0.11	0.11	0.10	0.10		
	28.88	0.10	0.10	0.09	0.09		
	57.76	0.09	0.09	0.09	0.09		

- 4 Days Degraded

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.16	0.14	0.13	0.14	$y = -0.047\ln(x) + 0.3404$	R2 = 0.997
	9.19	0.12	0.12	0.11	0.12		
	18.38	0.11	0.11	0.10	0.10		
	36.77	0.09	0.08	0.09	0.09		
50/44	3.72	0.00	0.00	0.00	0.00	$y = 0.3217x - 0.1786$	R2 = 0.9966
	9.30	0.12	0.13	0.15	0.13		
	18.59	0.10	0.12	0.11	0.11		
	37.19	0.09	0.10	0.10	0.10		
44/38	5.62	0.08	0.09	0.09	0.09	$y = 0.3806x - 0.2197$	R2 = 0.9999
	14.04	0.00	0.00	0.00	0.00		
	28.08	0.12	0.14	0.14	0.13		
	56.17	0.10	0.11	0.11	0.11		
38/32	6.93	0.09	0.10	0.09	0.09	$y = 0.2908x - 0.142$	R2 = 0.9928
	17.33	0.08	0.08	0.08	0.08		
	34.66	0.00	0.00	0.00	0.00		
	69.31	0.11	0.11	0.12	0.11		
38/44	5.78	0.10	0.10	0.10	0.10	$y = -0.047\ln(x) + 0.3404$	R2 = 0.9954
	14.44	0.09	0.08	0.09	0.09		
	28.88	0.09	0.08	0.08	0.08		
	57.76	0.00	0.00	0.00	0.00		

- **6 Days Degraded**

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.13	0.13	0.12	0.13	$y = 0.308x - 0.1595$	R2 = 0.9999
	9.19	0.12	0.11	0.10	0.11		
	18.38	0.10	0.10	0.09	0.10		
	36.77	0.09	0.09	0.08	0.09		
50/44	3.72	0.00	0.00	0.00	0.00	$y = 0.3089x - 0.1858$	R2 = 0.9972
	9.30	0.12	0.12	0.13	0.12		
	18.59	0.10	0.11	0.11	0.10		
	37.19	0.08	0.10	0.10	0.09		
44/38	5.62	0.07	0.09	0.08	0.08	$y = 0.3867x - 0.1723$	R2 = 0.9814
	14.04	0.00	0.00	0.00	0.00		
	28.08	0.14	0.15	0.15	0.14		
	56.17	0.12	0.13	0.13	0.13		
38/32	6.93	0.10	0.11	0.12	0.11	$y = 0.383x - 0.1777$	R2 = 0.9981
	17.33	0.09	0.10	0.11	0.10		
	34.66	0.00	0.00	0.00	0.00		
	69.31	0.14	0.14	0.13	0.14		
38/44	5.78	0.12	0.12	0.12	0.12	$y = 0.3522x - 0.1316$	R2 = 0.9863
	14.44	0.10	0.11	0.10	0.10		
	28.88	0.09	0.10	0.09	0.09		
	57.76	0.00	0.00	0.00	0.00		

- 8 Days Degraded

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.13	0.13	0.12	0.13	$y = -0.0497\ln(x) + 0.3481$	R2 = 0.9935
	9.19	0.12	0.11	0.10	0.11		
	18.38	0.10	0.10	0.09	0.10		
	36.77	0.09	0.09	0.08	0.09		
50/44	3.72	0.00	0.00	0.00	0.00	$y = 0.3179x - 0.2033$	R2 = 0.9981
	9.30	0.12	0.12	0.13	0.12		
	18.59	0.10	0.11	0.11	0.10		
	37.19	0.08	0.10	0.10	0.09		
44/38	5.62	0.07	0.09	0.08	0.08	$y = -0.0412\ln(x) + 0.351$	R2 = 0.9966
	14.04	0.00	0.00	0.00	0.00		
	28.08	0.14	0.15	0.15	0.14		
	56.17	0.12	0.13	0.13	0.13		
38/32	6.93	0.10	0.11	0.12	0.11	$y = 0.4041x - 0.2069$	R2 = 0.9979
	17.33	0.09	0.10	0.11	0.10		
	34.66	0.00	0.00	0.00	0.00		
	69.31	0.14	0.14	0.13	0.14		
38/44	5.78	0.12	0.12	0.12	0.12	$y = 2E-05x^2 - 0.0029x + 0.2904$	R2 = 0.9918
	14.44	0.10	0.11	0.10	0.10		
	28.88	0.09	0.10	0.09	0.09		
	57.76	0.00	0.00	0.00	0.00		

- 10 Days Degraded

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.12	0.14	0.15	0.14	$y = 0.3637x - 0.2281$	R2 = 0.9999
	9.19	0.11	0.11	0.12	0.11		
	18.38	0.09	0.09	0.11	0.09		
	36.77	0.08	0.08	0.09	0.08		
50/44	3.72	0.00	0.00	0.00	0.00	$y = -0.0389\ln(x) + 0.3056$	R2 = 0.9983
	9.30	0.14	0.13	0.12	0.13		
	18.59	0.12	0.11	0.11	0.11		
	37.19	0.10	0.10	0.10	0.10		
44/38	5.62	0.08	0.09	0.09	0.08	$y = 0.3581x - 0.1891x$	R2 = 0.9979
	14.04	0.00	0.00	0.00	0.00		
	28.08	0.12	0.14	0.13	0.13		
	56.17	0.10	0.12	0.12	0.11		
38/32	6.93	0.09	0.10	0.11	0.10	$y = 0.4145x - 0.2486$	R2 = 0.9949
	17.33	0.08	0.09	0.09	0.08		
	34.66	0.00	0.00	0.00	0.00		
	69.31	0.13	0.14	0.13	0.13		
38/44	5.78	0.11	0.10	0.10	0.10	$y = -0.0383\ln(x) + 0.3191$	R2 = 0.9945
	14.44	0.09	0.09	0.08	0.09		
	28.88	0.08	0.07	0.07	0.07		
	57.76	0.00	0.00	0.00	0.00		

- 12 Days Degraded

Sample	Pressure Kpa	Thickness (mm)				Model	R ²
		1	2	3	Average		
50/32	3.68	0.13	0.12	0.13	0.12	$y = 0.3097x - 0.1652$	R2 = 0.9896
	9.19	0.11	0.11	0.12	0.11		
	18.38	0.10	0.09	0.10	0.10		
	36.77	0.10	0.08	0.08	0.09		
50/44	3.72	0.12	0.12	0.13	0.12	$y = 0.3069x - 0.1868$ R2 = 0.9784	R2 = 0.9784
	9.30	0.10	0.11	0.11	0.11		
	18.59	0.08	0.09	0.10	0.09		
	37.19	0.08	0.08	0.09	0.08		
44/38	5.62	0.14	0.13	0.14	0.14	$y = 0.2775e^{-0.0084x}$	R2 = 0.9892
	14.04	0.12	0.12	0.13	0.12		
	28.08	0.11	0.11	0.12	0.11		
	56.17	0.06	0.10	0.11	0.09		
38/32	6.93	0.14	0.14	0.12	0.13	$y = 0.3609x - 0.1708$	R2 = 0.9725
	17.33	0.11	0.12	0.10	0.11		
	34.66	0.10	0.10	0.09	0.10		
	69.31	0.10	0.10	0.08	0.09		
38/44	5.78	0.14	0.13	0.12	0.13	$y = 0.3201x - 0.1354$	R2 = 0.9937
	14.44	0.12	0.11	0.11	0.11		
	28.88	0.10	0.10	0.10	0.10		
	57.76	0.09	0.10	0.10	0.09		

Appendix E: ANOVA Tables

Given in this appendix are the ANOVA table for compliance and elastic recovery

ANOVA for Compliance

Source	DF	Type I SS	Mean Square	F Value	Pr<F
ENDS	1	1.56	1.56	6.69	0.0109
PICKS	1	4.53	4.53	19.42	<0.0001
DAYS	1	0.76	0.76	3.26	0.0735
ENDS*PICKS	1	1.025	1.025	4.4	0.0382
ENDS*DAYS	1	0.49	0.49	2.11	0.149
PICKS*DAYS	1	0.05	0.05	2.22	0.6399

ANOVA for Elastic recovery

Source	DF	Type I SS	Mean Square	F Value	Pr<F
ENDS	1	1342.05	1342.05	25.49	<0.0001
PICKS	1	1.35	1.35	0.03	0.8728
DAYS	1	1050.45	1050.45	19.95	<0.0001
ENDS*PICKS	1	4.85	4.85	0.09	0.7671
ENDS*DAYS	1	27	27	0.51	0.475
PICKS*DAYS	1	0.52	0.52	0.01	0.9209