

Degradation Properties of Bioresorbable Material Candidates for Congenital Heart Defect
Repair

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Jessica Mae Holst

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Arthur G. Erdman

August 2013

© Jessica Holst 2013

Acknowledgements

I would like to sincerely thank Professor Erdman, Professor Carl Adams, Mike Brenzel, Paul Hindrichs, Zhengrong Zhou, Elli Käpylä, Sarath Eam, Stephanie Board, Dori Chretin, Jaishankar Kutty, Anna Stodolka, Matt Wolf, David and Rebecca Holst and Jared Savela for all the help, training and support I received for this project. Thanks are also in order to the Medical Industry Leadership Institute (MILI) Grant program at the Carlson School of Business and to St. Jude Medical for providing funding for this project.

I owe Jared Savela a distinct thank you for his help with data collection. A report that he generated, which consists mainly of organizing and presenting all the data for this project, is available in the supplementary materials of this thesis document.

Abstract

The goal of this project is to investigate the use of bioresorbable materials for congenital heart defect repair. This investigation focused on the in vitro degradation over eight weeks of several biodegradable polymers including: Poly (L-lactide) or PLLA, 70:30 Poly (L-lactide)-Polycaprolactone or PLLA/PCL and Polyglycolide or PGA. Since surface area can affect degradation rates several morphologies of these polymers were included in the study such as knits, films, felts, electrospun materials and sponges. The degradation was characterized by: tensile testing, scanning electron microscope (SEM) visualization, differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). Results of these tests do not directly provide recommendation for specific materials for use as implantable, bioresorbable materials but they do confirm that the combination of chemical composition and material morphology significantly affect the degradation rate as measured by changes in molecular weight with time. This finding supports the possibility of fine tuning manufacturing processes of these materials to obtain a specific degradation profile. PLLA film material 3 was the only material tested that maintained its peak stress and peak strain behavior over the 8 week degradation time period. This does not necessarily rule out the other materials as long as they maintain mechanical integrity over the required time period. In addition, the changes in molecular weight (but not stress and strain behavior) over time were significantly affected by the type of degradation environment the material was placed in, static vs. agitated or dynamic. The importance of the degradation rate and mechanism for this application is extremely important so the inclusion of some form of agitation in future degradation experiments is recommended. Finally, the degradation environment in this experiment was relatively inert and testing with digestive enzymes or other blood components is also recommended.

List of Tables	iiv
List of Figures.....	iv
Introduction.....	1
Characterizing the Cardiac Environment.....	2
Biomaterials Search and Selection.....	5
Biodegradation Study.....	6
Methods.....	12
Sample Preparation and Handling.....	12
Percent mass lost	13
Tensile Testing	13
Differential Scanning Calorimetry (DSC).....	13
Gel Permeation Chromatography (GPC)	15
Scanning Electron Microscopy (SEM)	15
Results.....	15
Initial Inspection and Qualitative Observation.....	15
Percent mass lost	16
Differential Scanning Calorimetry (DSC) Results.....	17
Scanning Electron Microscopy (SEM) Results.....	20
Gel Permeation Chromatography (GPC) Results and Analysis.....	45
GPC Data Analysis	46
Tensile Testing: Peak Strain Results and Analysis	48
Peak Strain Analysis.....	49
Tensile Testing: Peak Stress Results and Analysis	51
Peak Stress Analysis.....	52
Conclusions and Recommendations	54
References.....	57
Appendices.....	62
Appendix I: DSC.....	62
Appendix II: GPC Results.....	78
Appendix III: Peak Strain.....	100

List of Tables

Table 1: Tissue mechanical properties.....	4
Table 2: Degradation study material information.....	6
Table 3: Sample quantities and sample lengths for different types of testing.....	13
Table 4: Test method maximum temperature chart for DSC.....	14
Table 5: Values of the average T _m at baseline in degrees C.....	17
Table 6: Average T _g values at baseline (degrees C).....	18
Table 7: Average T _c values at baseline (degrees C).....	19
Table 8: Notable SEM results.....	20
Table 9: The average molecular weights of material samples at baseline.....	39
Table 10: Values of the average peak strain for each of the materials at baseline (Mpa).....	48
Table 11: Results of one-way ANOVAs of proportional peak strain by degradation time...	50
Table 12: Values of the average peak stress at baseline (Mpa).....	51
Table 13: Results of one-way ANOVAs of proportional peak stress by degradation.....	53

List of Figures

Figure 1: Materials 1, 2 & 3: PLLA Films.....	7
Figure 2: Material 4: PLLA Electrospun ‘BIOFELT’.....	7
Figure 3: Materials 5, 6 & 7: Electrospun PLLA/PCL.....	8
Figure 4: Materials 8, 9, 10, 11, 12 & 13: PLLA/PCL Films.....	9
Figure 5: Materials 14, 15, 16 & 17: PLLA/PCL Sponge.....	10
Figure 6: Material 18: PGA Warp Knit Mesh.....	11
Figure 7: Samples shown in testing vials.....	12
Figure 8: DSC preparation.....	14
Figure 9: Select examples of 8 week pH testing.....	15
Figure 10: Boxplot of the percent mass lost for each material/morphology category vs. time and environment.....	16
Figure 11: Boxplot of change in Tm from baseline in degrees C, plotted by morphology (material group)..	17
Figure 12: Boxplot of change in Tg from baseline in degrees C, plotted by morphology (material group)..	18
Figure 13: Boxplot of change in Tc from baseline in degrees C, plotted by morphology (material group)..	19
Figure 14: Baseline and 8 weeks agitated SEM images for PLLA film material 1 magnified 100 and 400 times.....	21
Figure 15: Baseline and 8 weeks agitated SEM images for PLLA film material 2 magnified 100 and 400 times.....	22
Figure 16: Baseline and 8 weeks agitated SEM images for PLLA film material 3 magnified 100 and 400 times.....	23
Figure 17: Baseline and 8 weeks agitated SEM images for PLLA BIOFELT material 4 magnified 100 and 400 times.....	24
Figure 18: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 5 magnified 100 and 400 times.....	25
Figure 19: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 6 magnified 100 and 400 times.....	26
Figure 20: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 7 magnified 100 and 400 times.....	27
Figure 21: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 8 magnified 100 and 400 times.....	28
Figure 22: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 9 magnified 100 and 400 times.....	29
Figure 23: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 10 magnified 100 and 400 times.....	30
Figure 24: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 11 magnified 100 and	

400 times.....	31
Figure 25: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 11 magnified 100 and 400 times.....	32
Figure 26: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 13 magnified 100 and 400 times.....	33
Figure 27: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 14 magnified 100 and 400 times.....	34
Figure 28: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 15 magnified 100 and 400 times.....	35
Figure 29: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 16 magnified 100 and 400 times.....	36
Figure 30: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 17 magnified 100 and 400 times.....	37
Figure 31: Baseline and 4 weeks agitated SEM images for PGA knit material 18 magnified 100 and 400 times.....	38
Figure 32: Boxplot of the proportion of the molecular weight at baseline by morphology, degradation time and degradation environment.....	45
Figure 33: Interval plot of the peak strain proportion of baseline peak strain.....	48
Figure 34: Interval plot of the peak stress proportion of baseline peak stress.....	51
Figure 35: Average change from baseline Tm for PLLA films and biofelt.....	62
Figure 36: Average change from baseline Tm for 70:30 PLLA/PCL sponges and PGA knit.....	63
Figure 37: Average change from baseline Tg for PLLA films and Biofelt.....	64
Figure 38: Average change from baseline Tg for electrospun 70:30 PLLA/PCL.....	65
Figure 39: Average change from baseline Tg of 70:30 PLLA/PCL films.....	66
Figure 40: Average change from baseline Tg for 70:30 PLLA/PCL sponges and PGA knit.....	67
Figure 41: Average change from baseline Tc for PLLA films and Biofelt samples.....	68
Figure 42: Average change from baseline Tc for 70:30 PLLA/PCL sponges and PGA knit samples.....	68
Figure 43: Individual value plot of the change in Tm by morphology, degradation time and degradation environment.....	69
Figure 44: Boxplot of the change in Tm by material, degradation time and degradation environment.....	70
Figure 45: Individual value plot of the change in Tm by material, degradation time and degradation environment.....	71
Figure 46: Individual value plot of the change in Tg by material, degradation time and degradation environment.....	72
Figure 47: Individual value plot of the change in Tg by morphology, degradation time and degradation	

environment.....	73
Figure 48: Boxplot of the change in Tg by material, degradation time and degradation environment.....	74
Figure 49: Individual value plot of the change in Tc by morphology, degradation time and degradation environment.....	75
Figure 50: Individual value plot of the change in Tc by material, degradation time and degradation environment.....	76
Figure 51: Individual value plot of the change in Tc by material, degradation time and degradation environment.....	77
Figure 52: Average percent of average molecular weight at baseline for PLLA samples.....	78
Figure 53: Average percent of average molecular weight at baseline for 70:30 PLLA/PCL electrospun samples.....	79
Figure 54: Average percent of average molecular weight at baseline for 70:30 PLLA/PCL film samples.....	80
Figure 55: Average percent of average molecular weight at baseline of 70:30 PLLA/PCL sponge samples.....	80
Figure 56: Interaction plot for the proportional molecular weight data, all materials.....	81
Figure 57: Boxplot of the proportion of molecular weight at baseline by material and degradation environment.....	90
Figure 58: Boxplot of the proportion of molecular weight at baseline by material and degradation time.....	90
Figure 59: Boxplot of the proportion of molecular weight at baseline by morphology and degradation time.....	91
Figure 60: Individual value plot of the proportion of the baseline molecular weight by morphology and degradation time.....	92
Figure 61: Interaction plot for the proportional molecular weight data, all materials.....	93
Figure 62: Residual plots for proportional molecular weight data, all materials.....	94
Figure 63: Interaction plot for the proportional molecular weight data, PLLA materials 1-4.....	95
Figure 64: Residual plots for the proportional molecular weight data, PLLA material 1-4.....	96
Figure 65: Interaction plots for the proportional molecular weight data, PLLA/PCL material 5-17.....	97
Figure 66: Residual plots for the proportional molecular weight data, PLLA/PCL material 5-17.....	98
Figure 67: Boxplot of proportional molecular weight by morphology, degradation time and degradation environment.....	99
Figure 68: Average percent change from baseline of average peak strain in PLLA films and biofelts.....	100
Figure 69: Average percent change from baseline of average peak strain for Electrospun 70:30 PLLA/PCL.....	101
Figure 70: Average percent change from baseline of average peak strain for 70:30 PLLA/PCL films.....	101
Figure 71: Average percent change from baseline of average peak strain of 70:30 PLLA/PCL sponges.....	

(materials 14 through 17) and PGA knit (material 18).....	102
Figure 72: Interaction plots for the proportional peak strain, all materials.....	102
Figure 73: Residual plots for the proportional peak strain, all materials.....	104
Figure 74: Interaction plot for proportional peak strain, materials 1-4.....	105
Figure 75: Residual plots for proportional peak strain, materials 1-4.....	106
Figure 76: Interaction plot for proportional peak strain, materials 5-17.....	107
Figure 77: Residual plots for proportional peak strain, materials 5-17.....	108
Figure 78: Interval plot of proportional peak strain vs material, degradation time and degradation environment.....	109
Figure 79: Residual plots for proportional peak strain, material 1.....	110
Figure 80: Residual plots for proportional peak strain, material 2.....	112
Figure 81: Residual plots for proportional peak strain, material 3.....	113
Figure 82: Residual plots for proportional peak strain, material 4.....	115
Figure 83: Residual plots for proportional peak strain, material 5.....	116
Figure 84: Residual plots for proportional peak strain, material 6.....	118
Figure 85: Residual plots for proportional peak strain, material 7.....	119
Figure 86: Residual plots for proportional peak strain, material 8.....	121
Figure 87: Residual plots for proportional peak strain, material 9.....	122
Figure 88: Residual plots for proportional peak strain, material 10.....	124
Figure 89: Residual plots for proportional peak strain, material 11.....	125
Figure 90: Residual plots for proportional peak strain, material 12.....	126
Figure 91: Residual plots for proportional peak strain, material 13.....	128
Figure 92: Residual plots for proportional peak strain, material 14.....	129
Figure 93: Residual plots for proportional peak strain, material 15.....	131
Figure 94: Residual plots for proportional peak strain, material 16.....	132
Figure 95: Residual plots for proportional peak strain, material 17.....	134
Figure 96: Average percent of baseline average peak stress in PLLA films and biofelt material (materials 1 through 4).....	134
Figure 97: Average percent of baseline peak stress in 70:30 PLLA/PCL electrospun materials.....	135
Figure 98: Average percent of average baseline peak stress in 70:30 PLLA/PCL film samples.....	135
Figure 99: Average percent of baseline average peak stress in 70:30 PLLA/PCL sponges and PGA knit.....	136
Figure 100: Interval plot of proportional peak stress by material, degradation time and degradation environment.....	137
Figure 101: Interaction plot of proportional peak stress, all materials.....	138
Figure 102: Residual plots of proportional peak stress, all materials.....	139

Figure 103: Interaction plot of proportional peak stress, materials 1-4.....	140
Figure 104: Residual plots of proportional peak stress, materials 1-4.....	141
Figure 105: Interaction plot of proportional peak stress, materials 5-17.....	142
Figure 106: Residual plots for proportional peak stress, materials 5-17.....	143
Figure 107: Residual plots for proportional peak stress, material 1.....	145
Figure 108: Residual plots for proportional peak stress, material 2.....	146
Figure 109: Residual plots for proportional peak stress, material 3.....	147
Figure 110: Residual plots for proportional peak stress, material 4.....	149
Figure 111: Residual plots for proportional peak stress, material 5.....	150
Figure 112: Residual plots for proportional peak stress, material 6.....	151
Figure 113: Residual plots for proportional peak stress, material 7.....	153
Figure 114: Residual plots for proportional peak stress, material 8.....	154
Figure 115: Residual plots for proportional peak stress, material 9.....	156
Figure 116: Residual plots for proportional peak stress, material 10.....	157
Figure 117: Residual plots for proportional peak stress, material 11.....	158
Figure 118: Residual plots for proportional peak stress, material 12.....	160
Figure 119: Residual plots for proportional peak stress, material 13.....	161
Figure 120: Residual plots for proportional peak stress, material 14.....	162
Figure 121: Residual plots for proportional peak stress, material 15.....	164
Figure 122: Residual plots for proportional peak stress, material 16.....	165
Figure 123: Residual plots for proportional peak stress, material 17.....	167

Introduction

In fetal circulation there is an opening between the atria in the heart allowing blood flow to bypass the lungs and this opening usually closes at birth. There are several types of congenital heart defects that occur when natural closing of this shunt between the atria fails. An atrial septal defect (ASD) is a congenital defect consisting of a physical hole between the atria and is usually immediately apparent. A patent foramen ovale (PFO) is a less obvious residual tunnel, or opening between flaps of tissue, between the atria. A PFO can have a 1 to 19 mm slit width (Hara et al., 2005; Kutty, Sengupta, & Khandheria, 2012) but the average PFO diameter is 3.4 mm in the first ten years of life and becomes 5.8 mm by approximately 100 years of age (Hara et al., 2005). Historically, PFO often goes undiagnosed but autopsy studies have shown that PFO is thought to be present in 9% to 25% of the adult population (Boucek, 2005; Fisher, 1995; Hara et al., 2005).

Recently and still controversial (Davis et al., 2013; Kutty et al., 2012), PFO has been linked to occurrences of cryptogenic stroke, migraine, visual aura and sleep apnea (Kutty et al., 2012; Mojadidi, Khessali, Gevorgyan, Levinson, & Tobis, 2012; Guchlerner et al., 2012). There are studies suggesting that closure of PFO has reduced the frequency and intensity of migraine (Rigatelli et al., 2012; Nagpal et al., 2013) as well as visual aura (Khessali, Mojadidi, Gevorgyan, Levinson, & Tobis, 2012; Mojadidi et al., 2012) but whether PFO closure is more effective than current treatments for cryptogenic stroke is still unclear (Kutty et al., 2012).

There are several transcatheter delivered medical devices that have been or are used to close congenital heart defects such as ASD and PFO. No devices are currently approved by the FDA for PFO closure and instead devices are used off label in these instances. Only two devices are approved for ASD closure as of 2012: the HELEXTM Septal Occluder and the AMPLATZERTM Septal Occluder (Medical policy: Closure devices for patent foramen ovale and atrial septal defects (ASD), 2012; The Cleveland Clinic

Foundation, 3/30/2012). The AMPLATZER™ Septal Occluder is made up of two disks connected by a waist, all made of nitinol metal mesh, and the disks contain polyester fabric (AMPLATZER™ septal occluder, 2012). The HELEX™ Septal Occluder is made of ePTFE (expanded polytetrafluoroethylene) and nitinol metal wire (GORE^(R) HELEX^(R) septal occluder, 2012; Proprietary ePTFE technology from gore, 2012).

Along with the possible health benefits mentioned previously there are health risks to having a permanently implanted device. These devices can be implanted at an early age and due to the relatively recent use of these devices long term studies are not available for permanently implanted materials in this location of the heart (Jux et al., 2002). In addition, non-bioresorbable materials do not grow with a patient's tissue (Jux et al., 2002). Permanently implanted materials in the atrial septum could also interfere with future use of non-invasive heart surgery techniques requiring trans-septal crossing (Jux, Bertram, Wohlsein, Bruegmann, & Paul, 2006), are a possible source of thrombosis and pose a risk of infection or tissue erosion (Jux et al., 2002).

It has also been noted that, "the device itself may be dispensable once the defect is closed and covered by a complete and firm layer of ingrown, endogenous "repair" tissue" (Jux et al., 2006). This may be especially appropriate in the case of PFO closure where there is no physical hole for tissue to grow into but a tunnel that needs to be closed (Jux et al., 2006). This would suggest the use of bioresorbable materials for PFO closure (Jux et al., 2002; Jux et al., 2006). A fully bioresorbable device may be ideal but the initial step of incorporating a bioresorbable fabric with a flexible metal frame seems a logical first step so only fabric biomaterials will be investigated in this study.

Characterizing the Cardiac Environment

If a device is to be placed within the extremely dynamic environment of the heart there are a number of environmental factors to be aware of when selecting a biomaterial fabric including but not limited to: atrial septal tissue histology, inter-atrial pressure

differences, atrial septal wall movement during the heartbeat cycle, tissue mechanical properties, immune response and tissue in-growth and endothelialization rates. These topics will be briefly discussed here.

Atrial Septal Tissue Histology: Long term implantation of a device in the heart, especially one that is meant to eventually degrade away, would optimally require an implanted material that mimics or is compatible with the existing tissue. There are always individual differences when it comes to anatomy but the atrial septum is primarily composed of muscle tissue along with amounts of fibrous and adipose tissue (Hara et al., 2005; Patten & Patten, 1931). At 1-2 months of age fibrous or connective tissue growth begins and by 8-9 months of age connective tissue has greatly increased with no significant difference in the amount of muscle tissue (Patten & Patten, 1931). These changes in tissue suggest that the natural mechanism of PFO closure utilizes connective tissue growth. Supporting this, case-by-case observations also support closure via fibrous tissue growth (Nir, Driscoll, & Edwards, 1994; Perloff, 1971; RAO & SISSMAN, 1971). In healthy individuals, the atrial surfaces such as the atrial septum are also covered with a layer of endothelial cells that act as a barrier between tissue and blood that prevents blood clots (Hara et al., 2005).

Inter-Atrial Motion and Pressure Differential: A medical device implanted within the atrial septum will be exposed to motion of the atrial septum as well as the inter-atrial pressure difference. Variations in anatomy allow for a large range of atrial septal motion but “a protrusion of the aneurysm >10 mm beyond the plane of the atrial septum into either the right or left atrium” is considered an atrial septal aneurysm in a study by Mügge et al. who noted that a specific definition is somewhat arbitrary and listed other definitions that have been used (Mügge et al., 1995). This group also noted that this definition is one of the broader, more inclusive definitions (Mügge et al., 1995). This would suggest that anything up to about 10 mm movement of the atrial septum is considered normal. However, atrial septal aneurysm is associated with atrial shunting, as

Mügge et al. reported that 106 of the 195 atrial septal aneurysm cases were concurrent with atrial shunting. This means that atrial septal deviations of about 18-23 mm may be involved in PFO closure (Mügge et al., 1995). In addition to movement, the device material and anchoring system must remain intact and seated in the atrial septum while experiencing a slight pressure differential between the atria. The pressure differential in a typical human heart is approximately in the order of 0-19 mmHg (Kupferschmid & Lang, 1983; Percy et al., 1985; Davidson et al., 2008).

Table 1: Tissue mechanical properties

Substance	Tensile Strength	Young's Modulus
Human cardiac tissue ^(Chen et al., 2008)	0.003-0.015MPa (3-15 kPa)	0.02 – 0.5 MPa
Fibrous Collagen ^(Chen et al., 2008)	1-7MPa	2-46 MPa
Collagen Gel (bovine) ^(Chen et al., 2008)	0.001-0.009 MPa (1-9 kPa)	0.002–0.022 MPa

Immune Response: Common immune system responses for current devices used for PFO and ASD closure in human case studies as well as animal studies were examined to provide a standard for comparison for any future work with biodegradable materials. In a study conducted by Sigler and Jux on looking at long term implantation of the Amplatzer and Starflex devices in animals and human case studies they report chronic mild inflammation associated with the polyester material of these devices “with some interindividual variation, the presence of multinucleated giant cells and lymphocytic infiltrates persisted and was observed also in the longest-term implants (1-year animal group, patient 12 after 4 years) without a tendency to diminish in its extent” (Sigler & Jux, 2007). Another study conducted by Jux et al. comparing the BioStar device made of heparin coated acellular collagen with the Starflex device had similar findings for the polyester material but “the BioSTAR matrix led to a transient, focally circumscribed

mild-to-moderate lymphocytic immune response” (Jux et al., 2006). This finding would support the use of biocompatible and biodegradable materials for congenital heart defect repair.

Tissue in-growth: Tissue in-growth and endothelialization rates of current devices in human case studies and animal studies were also investigated to use as a standard for comparison. In general, studies report substantial endothelialization and tissue ingrowth at time points around thirty to ninety days (Jux et al., 2006; Sigler & Jux, 2007; Zahn, Wilson, Cutright, & Latson, 2001). The study by Sigler and Jux also noted that complete endothelialization of devices (including the metal frames) is approximately three to five months after implantation (Sigler & Jux, 2007). However, Chessa and colleagues share a case study where one side of an Amplatzer device remained uncovered by an endothelial cell layer after approximately two years implantation (Chessa, Butera, Frigiola, & Carminati, 2004). Studies involving the BioStar degradable device show that “longitudinal splits and fraying at the terminal edges of the collagen matrix allowed for infiltration of the matrix with fibroblastic and monoclear lymphoid cells” at approximately thirty days after implantation and “calculated that approximately 90% of all implanted ICL material had been resorbed after two years in vivo” (Jux et al., 2002). Taken together, these findings support the idea that a degradable material will allow for tissue ingrowth and this ingrowth, and corresponding partial material degradation, should occur within the first thirty days after implantation. It is also important that the septum and bioresorbable device material retain their integrity until complete endothelialization and tissue ingrowth, somewhere on the order of five to twenty-four months.

Biomaterials Search and Selection

Materials selection began with an literature search of bioresorbable materials candidates and after an initial search there were a number of bioresorbable materials considered for this study including: manufactured collagens, Poly (L-lactide) (PLLA), poly(lactic-co-

glycolic acid) (PLGA), Poly(ester-urethane) (PEUUs), Poly (L-lactide)-Polycaprolactone (PLLA/PCL), PLGA combinations with PLLA fabric and Polyglycolide (PGA).

The materials previously listed were further investigated with a focus on the compatibility with the environmental characteristics discussed above. Next a meeting was held with a medical device company that this study partnered with. The material selection process focused on degradation characteristics, cytotoxicity/immune response, material properties, current FDA standing and manufacturability/obtainability of the bioresorbable materials listed above. Within that meeting three materials were selected for biodegradation testing: Poly (L-lactide) (PLLA), Poly (L-lactide)-Polycaprolactone (PLLA/PCL) and Polyglycolide (PGA). In addition to these three chemical compositions, several morphologies of the first two of these materials were selected to determine how tunable the degradation characteristics were by the slight variations in surface area resulting from different manufacturing processes.

Biodegradation Study

The heart is such a dynamic environment there was concern that fluid flow rates/agitation may affect degradation so two environmental conditions were selected for this study. The first is a stagnant environment (considered a fairly standard condition for degradation tests and complies with ASTM standards) and an agitated condition where sample vials were placed in an incubator shaker set at 80 rpm. The time points of interest were 2 weeks, 4 weeks, and 8 weeks which were meant to correspond to particular points of endothelialization and cell in-growth behavior. The following items were recorded for each of the materials at the different degradation time points and, where appropriate, compared to data collected at a baseline for each material. Another option for the partnering medical device company would be to look at the behavior of their Angioseal Anchor medical device in the specific conditions performed in this study and compare

that to its known behavior in vivo as they have access to data on its in vivo performance and can subject it to the same in vitro conditions of our study for comparison.

Degradation Characterization Measures:

1. Mass Measurement – the percent of mass lost relative to initial mass provides “macro” level degradation characterization
2. Tensile Testing - peak stress (or ultimate stress) and peak strain (engineering stress and strain) provides information on if and how mechanical properties of the materials change with degradation
3. Gel Permeation Chromatography (GPC) - molecular weight measurement provides molecular level degradation characterization
4. Differential Scanning Calorimetry (DSC) – measures of glass transition, melting and crystallization temperatures (T_g , T_m , and T_c respectively) provides information on how molecular weight change relates to physical properties of materials and insight into molecular bonding behavior
5. Scanning Electron Microscopy (SEM) - used for visual characterization of surface degradation

Table 2: Degradation study materials information

Material	Manufacturer	Composition	Type	Pore Size	Porosity	Thickness
PLLA Candidates						
1	Proxy	PLLA (Poly (L-lactide))	Film	0.3 mm	15%	0.14mm
2	Proxy	PLLA (Poly (L-lactide))	Film	0.35 mm	20%	0.11mm
3	Proxy	PLLA (Poly (L-lactide))	Film	0.7 mm	50%	0.20mm
4	Concordia	PLLA (Poly (L-lactide))	BIOFELT	N/A	(200 mg/cc)	0.3 mm
PLLA/PCL Candidates						
5	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Electrospun	0.6 mm	40%	~0.35mm
6	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Electrospun	N/A	65%	0.08mm
7	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Electrospun	0.5 mm	70%	0.08mm
8	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.65 mm	40%	0.11mm
9	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.7 mm	50%	0.08mm
10	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.75 mm	55%	0.11mm
11	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.5 mm	55%	0.16mm
12	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.75 mm	60%	0.08mm
13	Proxy	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Film	0.55 mm	70%	0.14mm
14	Kensey-Nash	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Sponge	N/A	0.45 g/cm ³	0.27 mm
15	Kensey-Nash	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Sponge	N/A	0.73 g/cm ³	0.24 mm
16	Kensey-Nash	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Sponge	N/A	0.70 g/cm ³	0.16 mm
17	Kensey-Nash	70:30 PLLA/PCL (Poly (L-lactide)-Polycaprolactone)	Sponge	N/A	0.43 g/cm ³	0.15 mm
PGA Candidate						
18	Concordia	Polyglycolide (PGA)	Knit	N/A	N/A	~0.21 mm

Materials 1, 2 & 3: PLLA Films

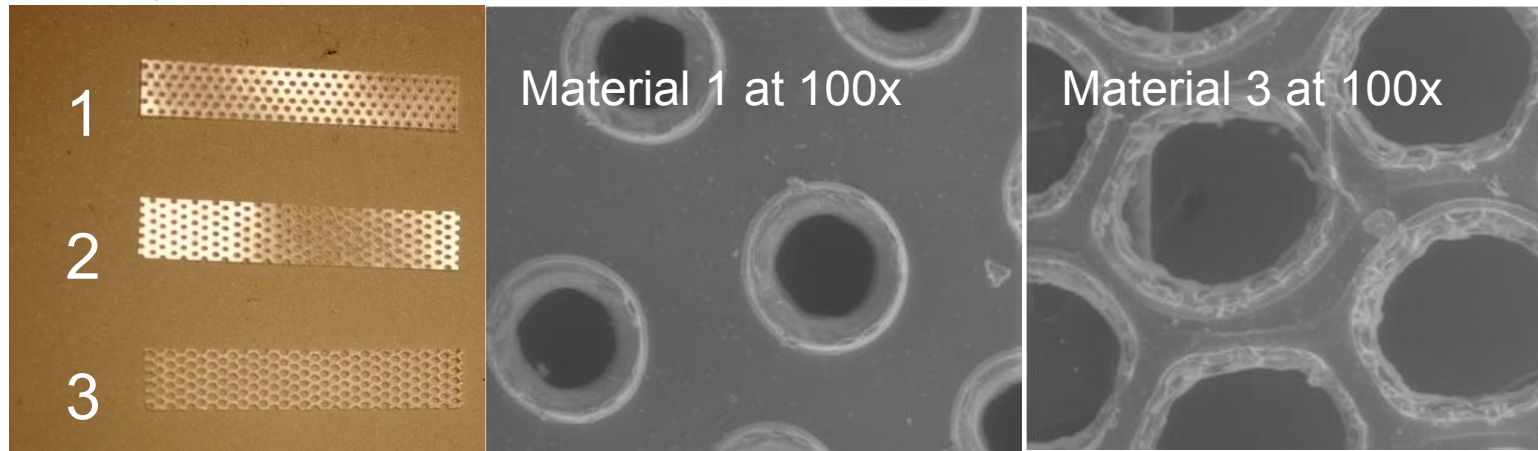


Figure 1: Material 1: Proxy PLLA Film - 0.3mm pore diameter, 15% porosity, 0.14mm thickness
Material 2: Proxy PLLA Film - 0.35mm pore diameter, 20% porosity, 0.11mm thickness
Material 3: Proxy PLLA Film - 0.7mm pore diameter, 50% porosity, 0.20mm thickness

Material 4: PLLA Electrospun 'BIOFELT'

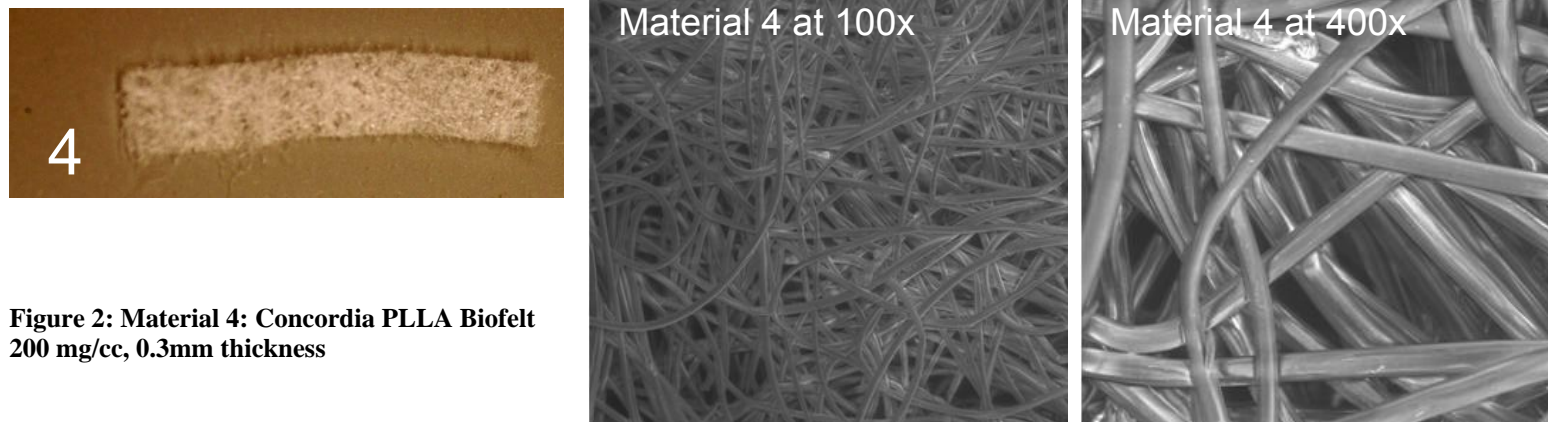


Figure 2: Material 4: Concordia PLLA Biofelt
200 mg/cc, 0.3mm thickness

Materials 5, 6 & 7: Electrospun PLLA/PCL

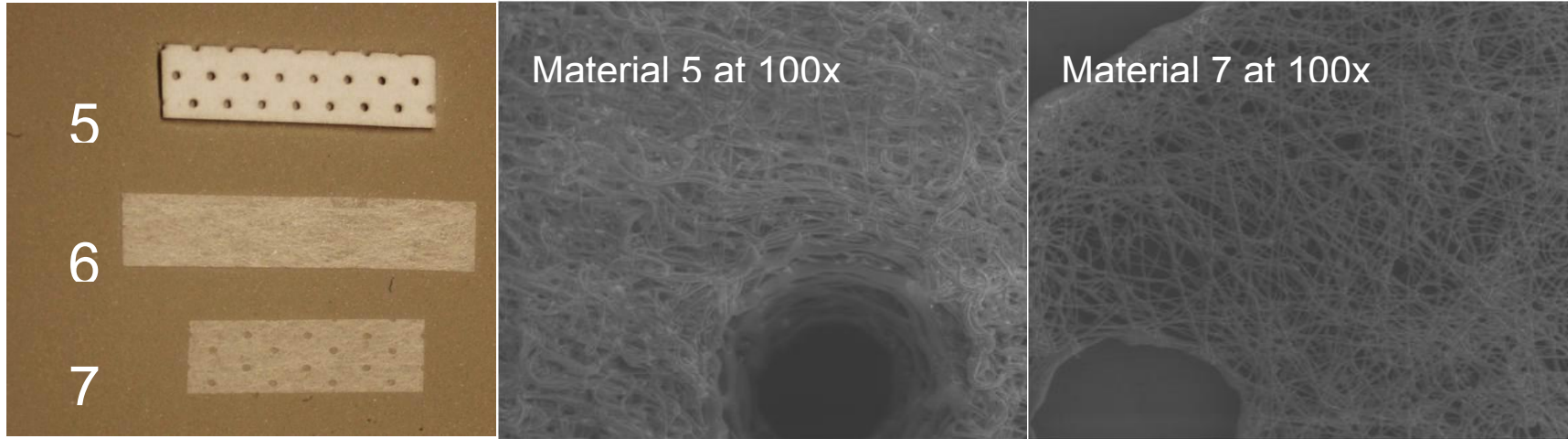
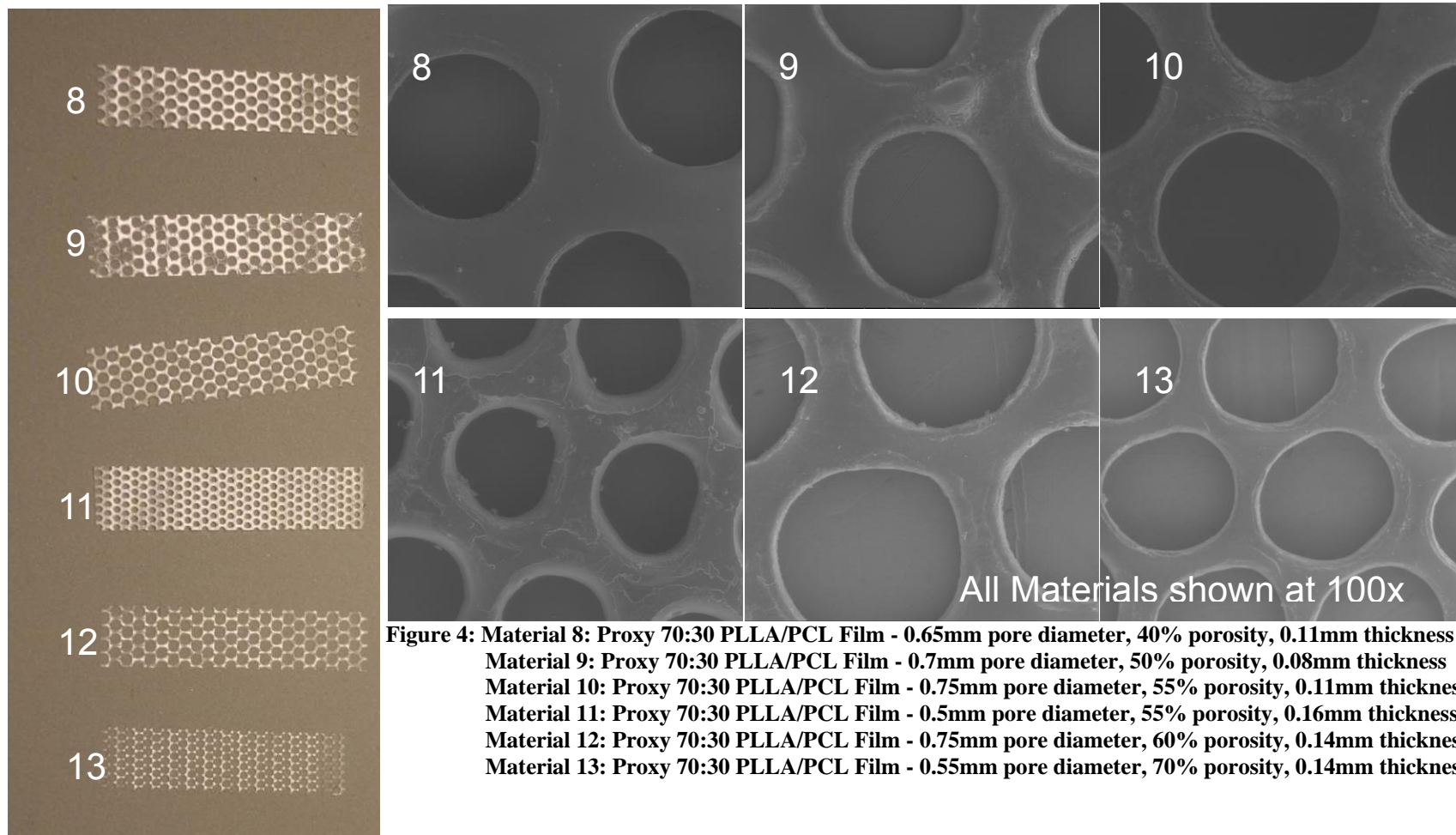


Figure 3: Material 5: Proxy Electrospun 70:30 PLLA/PCL - 0.6mm pore diameter, 40% porosity, 0.35mm thickness
Material 6: Proxy Electrospun 70:30 PLLA/PCL - no pores, 65% porosity, 0.08mm thickness
Material 7: Proxy Electrospun 70:30 PLLA/PCL - 0.5mm pore diameter, 70% porosity, 0.08mm thickness

Materials 8, 9, 10, 11, 12 & 13: PLLA/PCL Films



**Figure 4: Material 8: Proxy 70:30 PLLA/PCL Film - 0.65mm pore diameter, 40% porosity, 0.11mm thickness
Material 9: Proxy 70:30 PLLA/PCL Film - 0.7mm pore diameter, 50% porosity, 0.08mm thickness
Material 10: Proxy 70:30 PLLA/PCL Film - 0.75mm pore diameter, 55% porosity, 0.11mm thickness
Material 11: Proxy 70:30 PLLA/PCL Film - 0.5mm pore diameter, 55% porosity, 0.16mm thickness
Material 12: Proxy 70:30 PLLA/PCL Film - 0.75mm pore diameter, 60% porosity, 0.14mm thickness
Material 13: Proxy 70:30 PLLA/PCL Film - 0.55mm pore diameter, 70% porosity, 0.14mm thickness**

Materials 14, 15, 16 & 17: PLLA/PCL Sponge

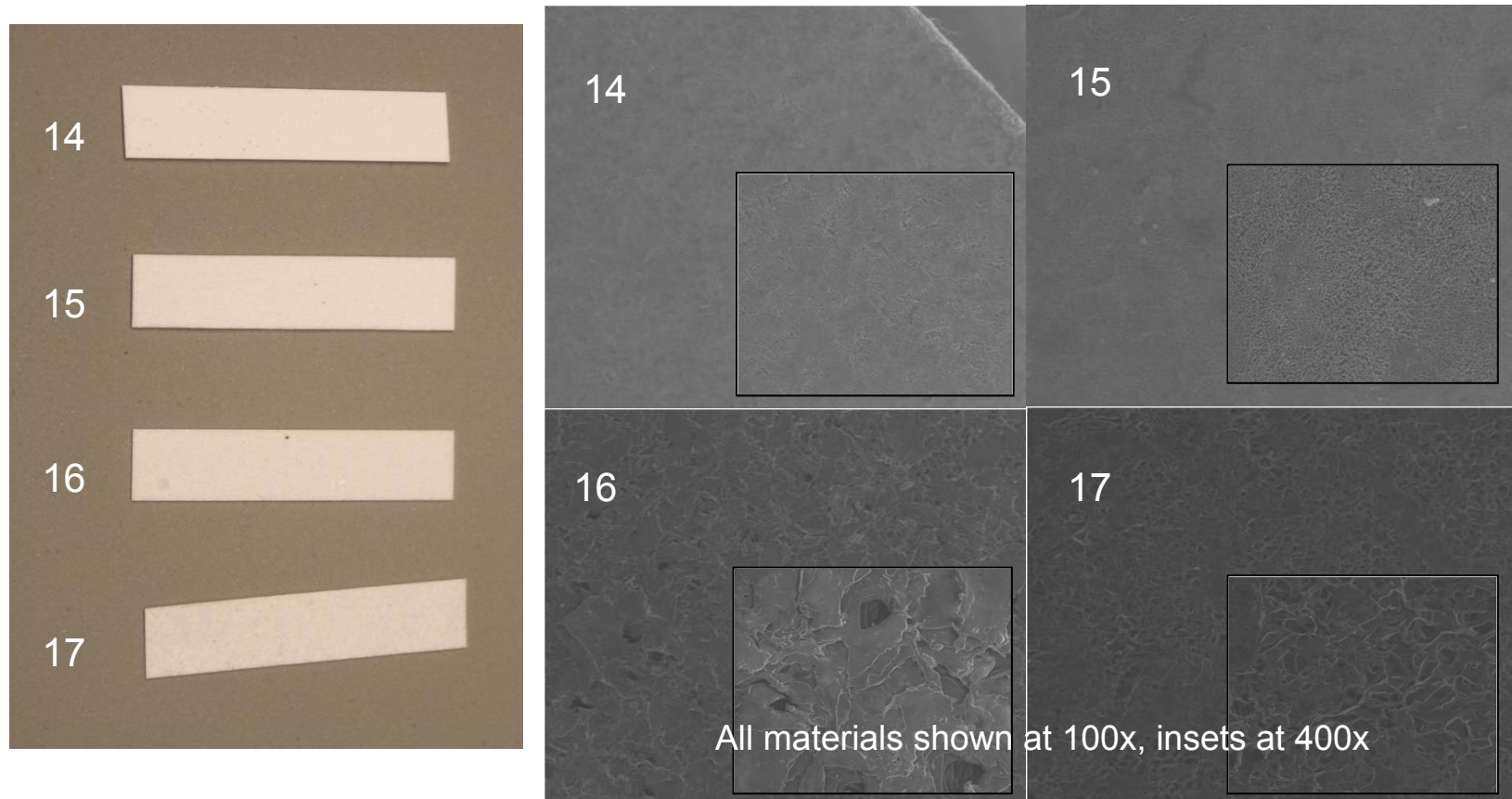


Figure 5: Material 14: Kensey-Nash PLLA/PCL Sponge - 0.27mm thickness, 0.45g/cm³
Material 15: Kensey-Nash PLLA/PCL Sponge - 0.24mm thickness, 0.73g/cm³
Material 16: Kensey-Nash PLLA/PCL Sponge - 0.16mm thickness, 0.70g/cm³
Material 17: Kensey-Nash PLLA/PCL Sponge - 0.15mm thickness, 0.43g/cm³

Material 18: PGA Warp Knit Mesh

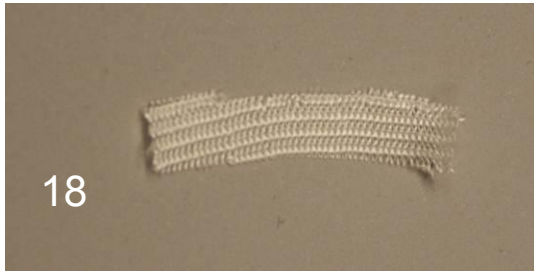
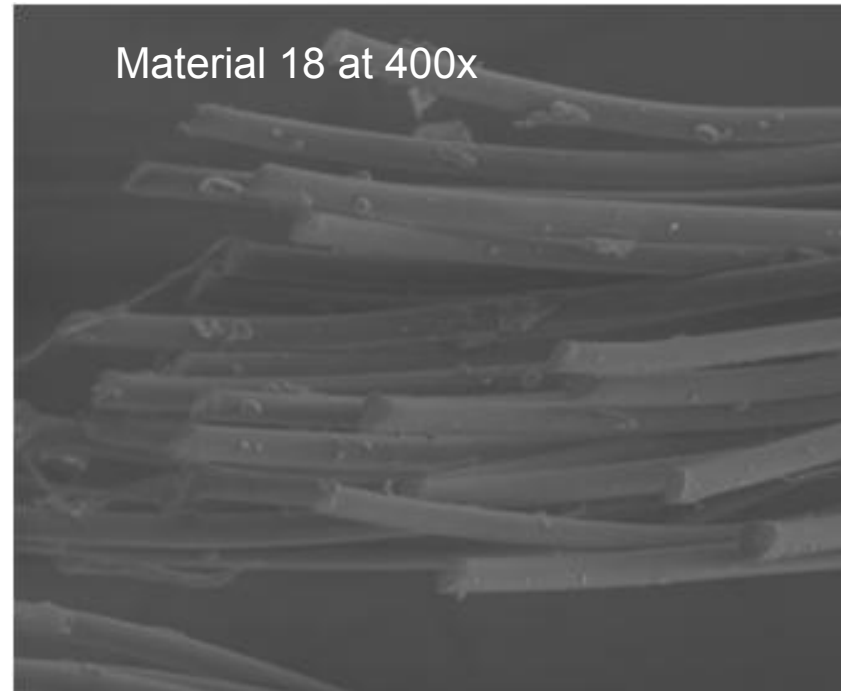
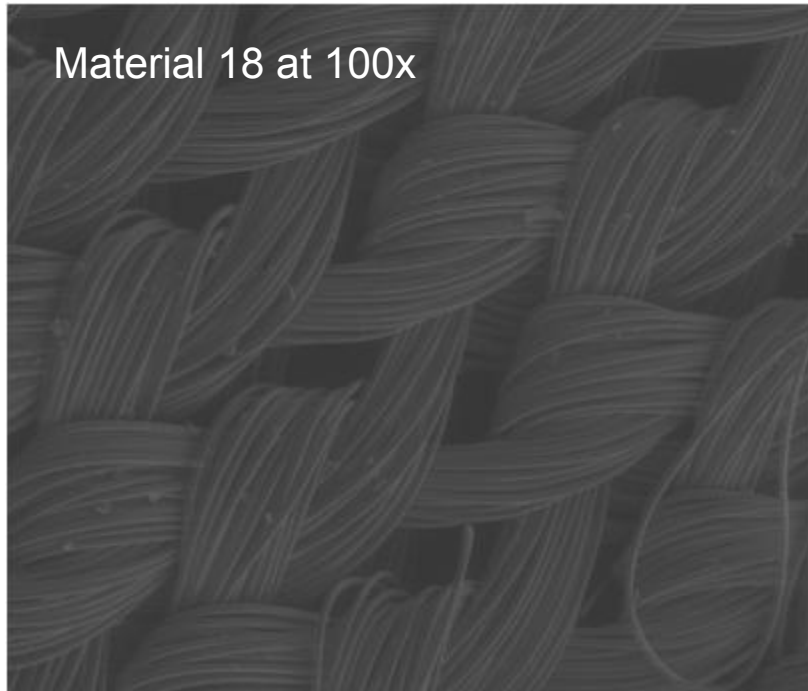


Figure 6: Material 18: Concordia PGA Warp Knit Mesh - approximately 0.02mm thickness, Knit pattern: 1-0-1/1-2-1



Methods

Sample Preparation and Handling

An effort was made to minimize the amount of time the samples spent in an open air environment between opening the packaging and placing the samples in solution to minimize exposure to environmental moisture.

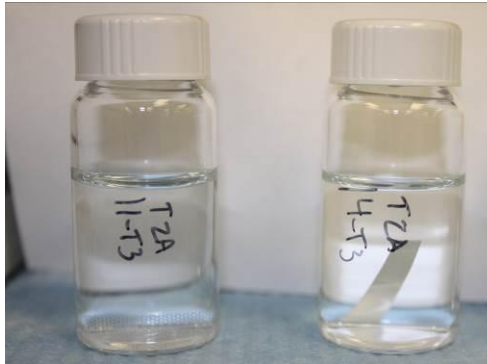


Figure 7: Samples shown in testing vials

Gloves were worn by the investigators while handling the samples to protect them from skin oils or other contaminants. Vials (20 mL) of the pH buffered saline solution (0.01 M phosphate buffer and 0.01 NaCl with pH of 7.4) were prepared and labeled with a marker. Next the sample packaging was opened and the samples were cut with two special rolling cutting devices (4mm and 20mm widths) that could make consistent cuts so that the samples were uniform sizes. Once cut, samples (sample mass ranging from approximately 0.012g to 0.015g) were placed in the vials, submerged in the solution, and sealed by screwing on the cap. Half of these samples were then placed in an incubator at body temperature ($37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), the stagnant degradation environment (environment 1), and the other half of the samples were placed in an incubator/agitator at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and at 80 RPM, the agitated degradation environment (environment 2). At each time point (2 weeks, 4 weeks, and 8 weeks) the appropriate samples were removed from the buffered solution, rinsed with de-ionized water, placed in a cassette, dried in a Lyophilizer overnight and frozen (if testing did not occur) immediately to arrest any further degradation. The pH of one sample from each of the materials was tested periodically (approximately every two weeks) with pH indicator strips (colorpHast by EMD) and if the solution was too acidic (pH of 6 or less) more buffered saline solution was added to the corresponding group of samples.

Table 3: Sample quantities and sample lengths for different types of testing (for each material)

	Tensile (30 mm)	GPC (20 mm)	DSC (20 mm)	SEM (10 mm)
Agitated	3	3	3	2
Stagnant	3	3	3	2
# samples for each material and each test	6	6	6	4
# samples for all time periods	24	24	24	16
Total # of samples for each material	88			

Percent mass lost

The initial mass of 3 samples from each of the two environments at all three time points were recorded with a digital scale (mg). At each time point the appropriate samples were removed, dried and frozen. Then, each sample was re-weighed on the digital scale (mg). The percent mass lost relative to the initial, or baseline mass, of each individual sample is then calculated.

Tensile Testing

Procedure: Samples soak in DI water for several minutes, are loaded into and Instron Tensile Tester 5542 (Mitutoyo 12 mm gauge length) with sandpaper enhanced grips and pulled at a rate of 20 mm/min until failure. Note: Due to size constraints 30 mm x 4 mm pieces of material were used in tensile testing.

Differential Scanning Calorimetry (DSC)

Samples for DSC were cut into small squares and as many as would fit were placed in the standard aluminum pans (goal weight between 3mg and 5mg) and used for DSC testing. The samples were then run with the TA Instruments Thermal Analysis - DSC Standard Cell RC with the appropriate profile for each material composition (see Table 3 below). Three readings were recorded for each material at each time point.

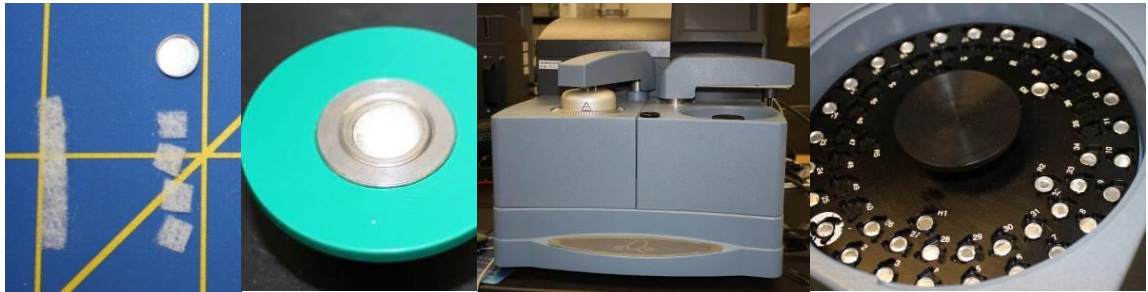


Figure 8: DSC preparation Far left – cutting the sample into test pieces
 Second to left – sample in the container (ready for the lid)
 Second from right – Calorimeter
 Far Right – samples capped and loaded for testing

DSC Test Method: (for actual values of MAX TEMP for each material see chart below)

- 1: Equilibrate at -90.00 °C
- 2: Ramp 20.00 °C/min to MAX TEMP °C
- 3: Mark end of cycle 0
- 4: Ramp 20.00 °C/min to -90.00 °C
- 5: Mark end of cycle 0
- 6: Ramp 20.00 °C/min to MAX TEMP °C
- 7: Mark end of cycle 0

Table 4: Test method maximum temperature chart for DSC

Material #s	Composition	Type	Max DSC Temp
1-3	PLLA	Film	220°C
4	PLLA	BIOFELT	220°C
5-7	PLA-PCL	Electrospun	200°C
8-13	PLA-PCL	Film	200°C
14-17	PLLA-PCL	Sponge	220°C
18	PGA	Warp Knit Mesh	260°C

Gel Permeation Chromatography (GPC)

After individual mass measurements were made with a digital scale, the samples for GPC were dissolved in the appropriate amount of chloroform to make a 1mg/ml solution. The samples were then agitated to completely dissolve the samples in the chloroform. The GPC samples were then run with 3 tests from each vial (3 tests for each material). Polystyrene standards were used to generate the calibration curve relating retention time to estimated molecular weight.

Scanning Electron Microscopy (SEM)

SEM photos were taken using a FEI 400 Scanning Electron Microscope on low vacuum setting with the voltage set to 5-10kV. The samples were placed on the SEM stage using carbon fiber tape. Initially the samples were viewed at low magnification (20-50x) to look for major signs of material breakdown. Images of significant features were recorded. At least one image of each material was recorded at 100x and at least 3 points were recorded at 400x.

Results

Initial Inspection and Qualitative Observation

pH observations: Most of the samples stayed within the desired pH range but many were close to a pH of 5 at the final measurement recorded as the samples were ‘harvested.’ Material 18 was particularly acidic and needed buffer solution several times to keep it within the desired pH range and went out of the desired range at the by the end of the last degradation period.

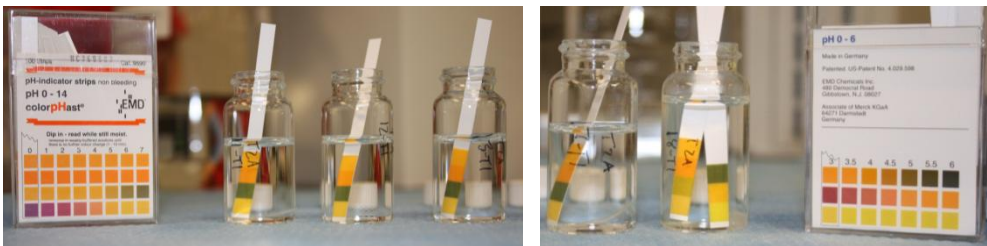


Figure 9: Select examples of 8 week pH testing – materials shown above from left to right 11, 12, 13, 17 and 18

Visual Inspection: All but the material 18 samples looked relatively similar to the initial samples to the unaided eye. The material 18 samples however, completely frayed into small fragments or fibers that were extremely difficult to collect. The fibers stuck to filter paper or were completely

blown away by the slightest air current. Attempts to centrifuge the fibers from the solution also failed.

Percent mass lost

Upon initial inspection of the degradation data of all 18 materials, it appears compromised as a number of samples actually gained mass (a negative % mass lost on the graphic below) over time. It is hypothesized that due to time constraints of having ‘harvested’ a large quantity of samples at one time period, attempts to get them rinsed and dried in a short window of time may have allowed deposits from the buffer solution to remain on/in the samples due to incomplete rinsing. The possible incomplete rinsing issue was not apparent until after testing. Also, the PGA percent mass loss may be incorrectly high for the last few weeks as the material did not completely dissolve in the solution but much of it was suspended as fibers. These fibers could not be separated from the solution despite attempts to centrifuge them apart. PGA percent mass loss for the 8 week samples could not be measured at all because the fabric completely frayed.

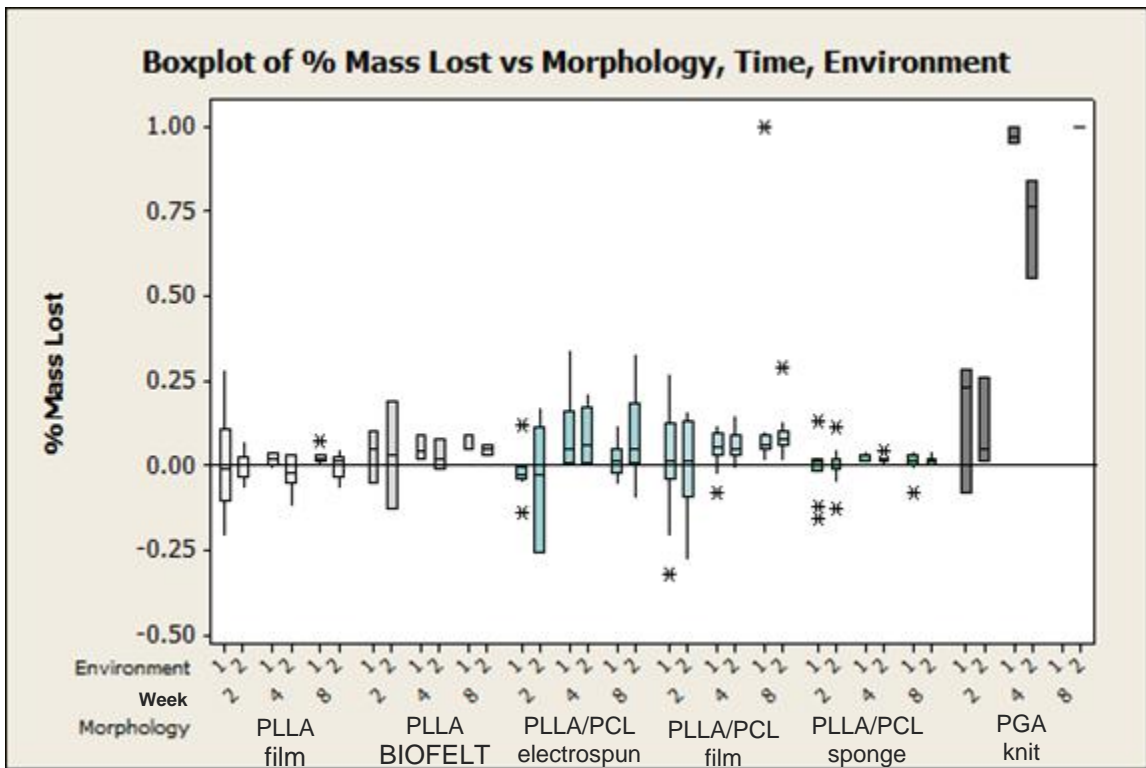


Figure 10: Boxplot of the percent mass lost for each material/morphology category vs. time and environment

Differential Scanning Calorimetry (DSC) Results

Table 5: Values of the average Tm at baseline in degrees C, note: materials 16 and 17 have no baseline data (graphs show baseline = 2wks stagnant data for material 16 and 17)

Average Tm at Baseline (degrees C)			
Material	Average	Material	Average
1-BL	176.98	14-BL	160.74
2-BL	176.49	15-BL	159.89
3-BL	176.79	16- 2 weeks*	158.97
4-BL	176.90	17- 2 weeks*	158.45
		18-BL	219.49

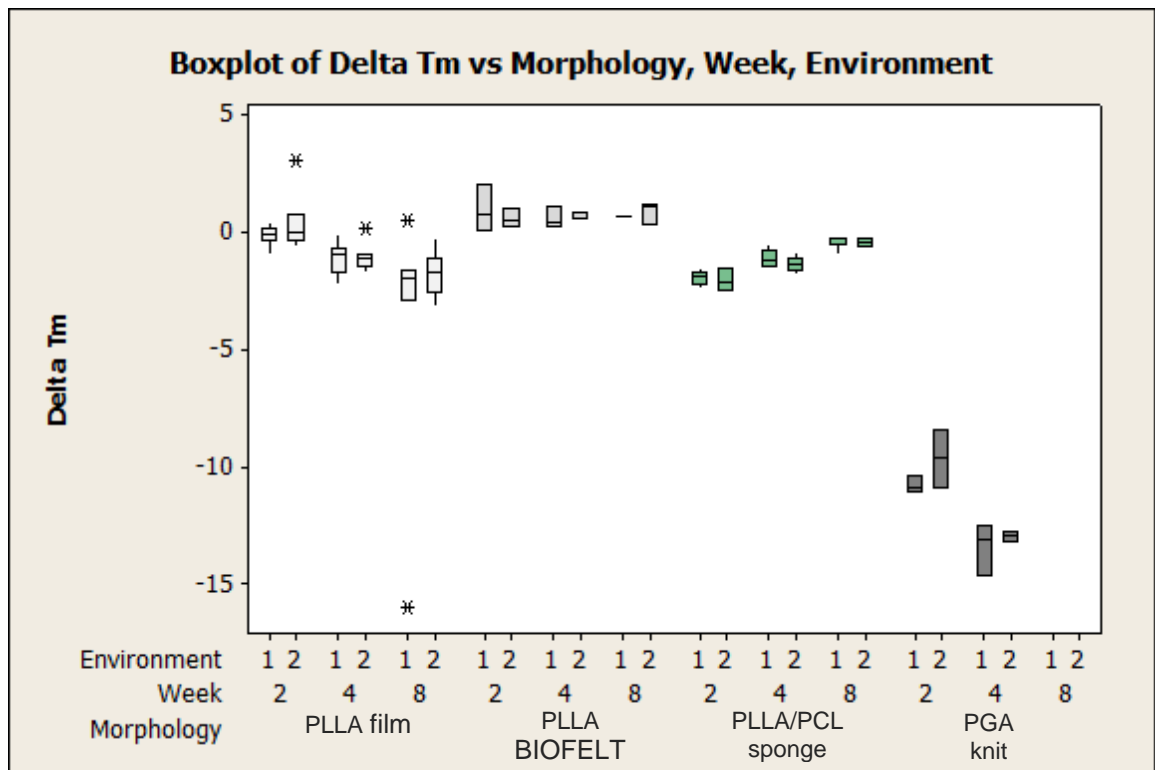


Figure 11: Boxplot of change in Tm from baseline in degrees C, plotted by morphology (material group) with materials 16 and 17 removed

Table 6: Average Tg values at baseline (degrees C)

Average Tg at Baseline (degrees C)			
Material	Average	Material	Average
1-BL	59.50	10-BL	18.81
2-BL	58.86	11-BL	19.34
3-BL	59.41	12-BL	20.32
4-BL	60.31	13-BL	18.96
5-BL	19.52	14-BL	38.65
6-BL	20.09	15-BL	37.90
7-BL	20.10	16- 2 weeks*	27.30
8-BL	19.40	17- 2 weeks*	33.40
9-BL	21.08	18-BL	38.45

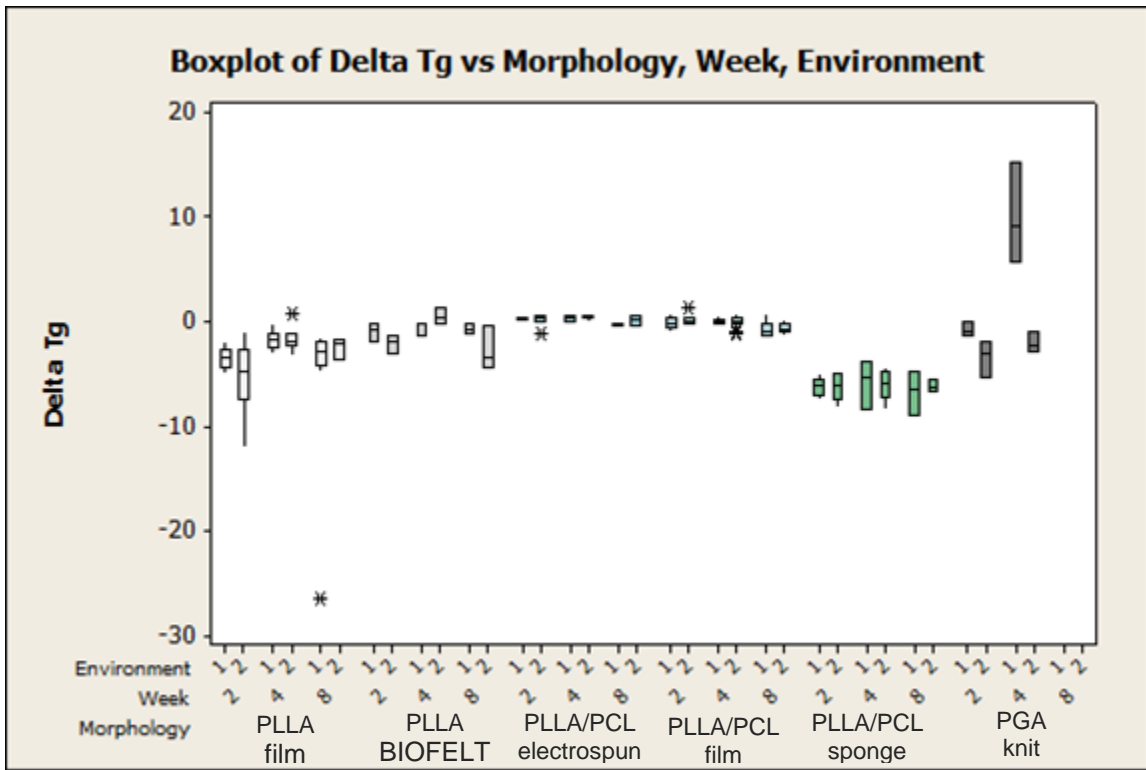


Figure 12: Boxplot of change in Tg from baseline in degrees C, plotted by morphology (material group) with materials 16 and 17 removed

Table 7: Average Tc values at baseline (degrees C) *values reported here for baseline 16, 17 and 18 are from the 2 week data

Average Tc at Baseline (degrees C)			
Material	Average	Material	Average
1-BL	133.09	15-BL	120.97
2-BL	123.96	16- 2 weeks*	125.22
3-BL	132.20	17- 2 weeks*	124.58
4-BL	108.26	18- 2 weeks*	101.38
14-BL	125.21		

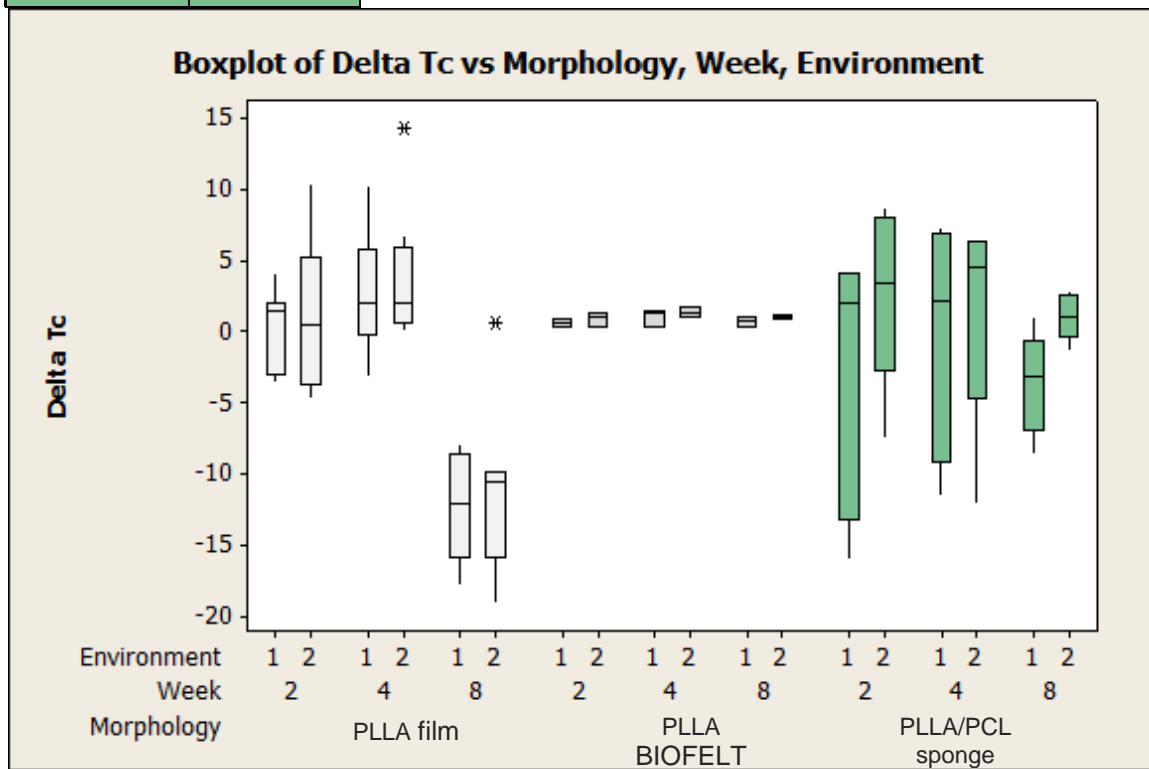


Figure 13: Boxplot of change in Tc from baseline in degrees C, plotted by morphology (material group) *Note that materials 16, 17 and 18 are not plotted due to missing baseline values

No statistical analysis was performed on DSC data because not every set of sample groups contains three measures due to equipment error. Results for melting temperature (Tm) suggest slight change over time but no material changed more than 10% from the corresponding baseline measures. With the exception of a couple of measures, the glass transition temperature (Tg) remained within 10% of baseline except for materials 14 and

15 which appear to hover around 20% of baseline and the 4 weeks stagnant group of material 18 which increased 20-40%. The crystallization temperature (Tc) of the materials suggest slight change over time, particularly the PLLA film materials between 4 and 8 weeks, but no material was more than 15% different from baseline measures.

Scanning Electron Microscopy (SEM) Results

Overall, there was not any major damage (fractures/cracks/etc.) recorded in the scanning electron microscopy images (with the exception of material 18). The following is a selection of representative images at 100x and 400x magnification of each of the 18 materials at baseline and at 8 weeks agitated (except for material 18 which is baseline and 4 weeks agitated).

Table 8: Notable SEM results

PLLA materials 1 through 3 had no visible changes
The fibers of the PLLA BIOFELT appeared to swell and slightly spread out at 8 weeks degradation.
The fibers of the electrospun PLLA/PCL materials 5, 6 and 7 appeared to spread slightly by 8 weeks degradation.
The surface of the PLLA/PCL films (materials 8 through 13) appears rougher at 8 weeks degradation.
Materials 14 through 17, the PLLA/PCL sponges all appear rougher at 8 weeks degradation and small, concentrated pockets of material appear to be missing on material 14.
PGA material 18 completely unraveled by 8 weeks. At 4 weeks the distinct knit pattern was almost completely disrupted.

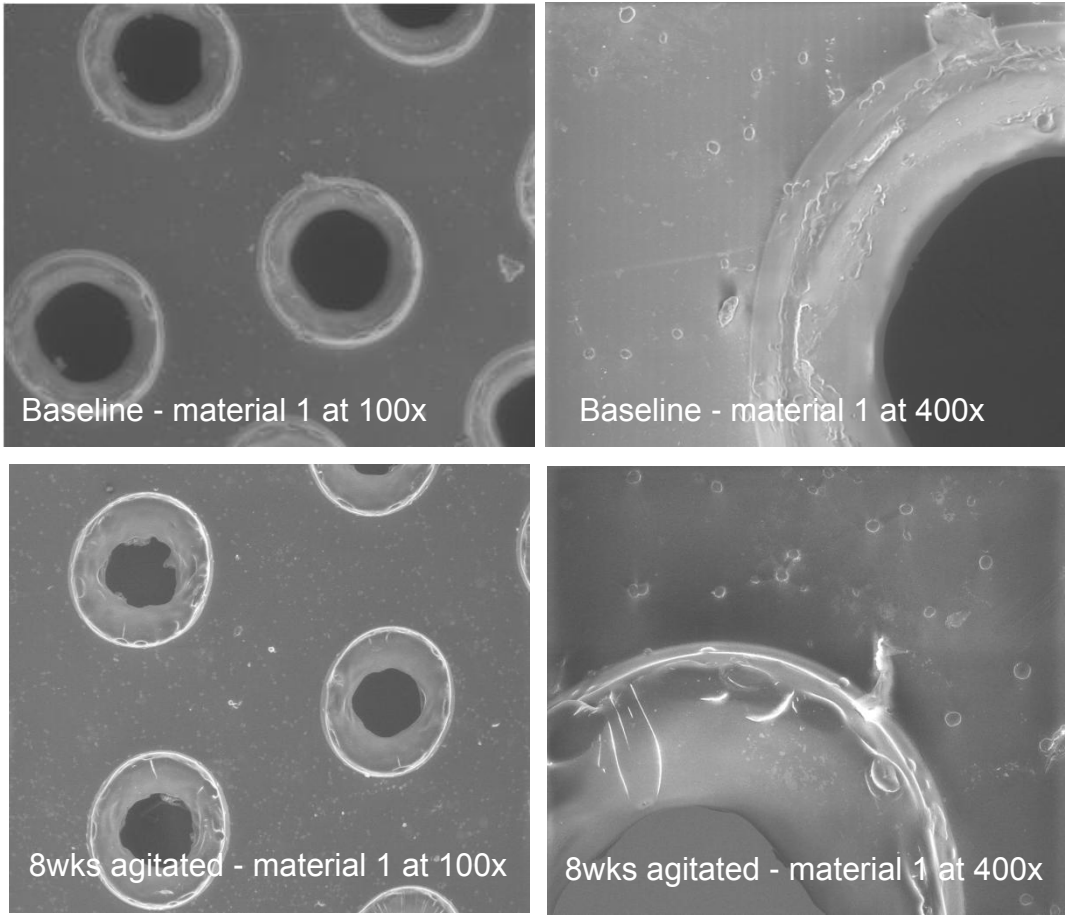


Figure 14: Baseline and 8 weeks agitated SEM images for PLLA film material 1 magnified 100 and 400 times

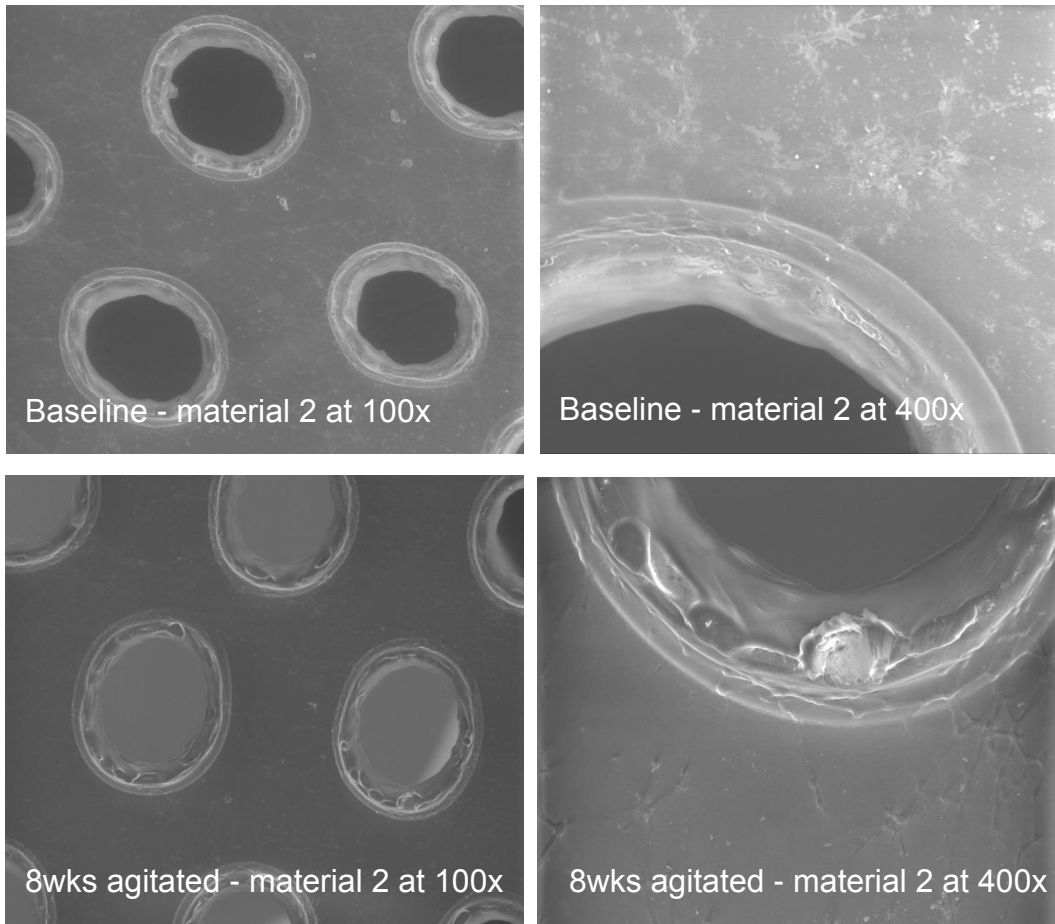


Figure 15: Baseline and 8 weeks agitated SEM images for PLLA film material 2 magnified 100 and 400 times

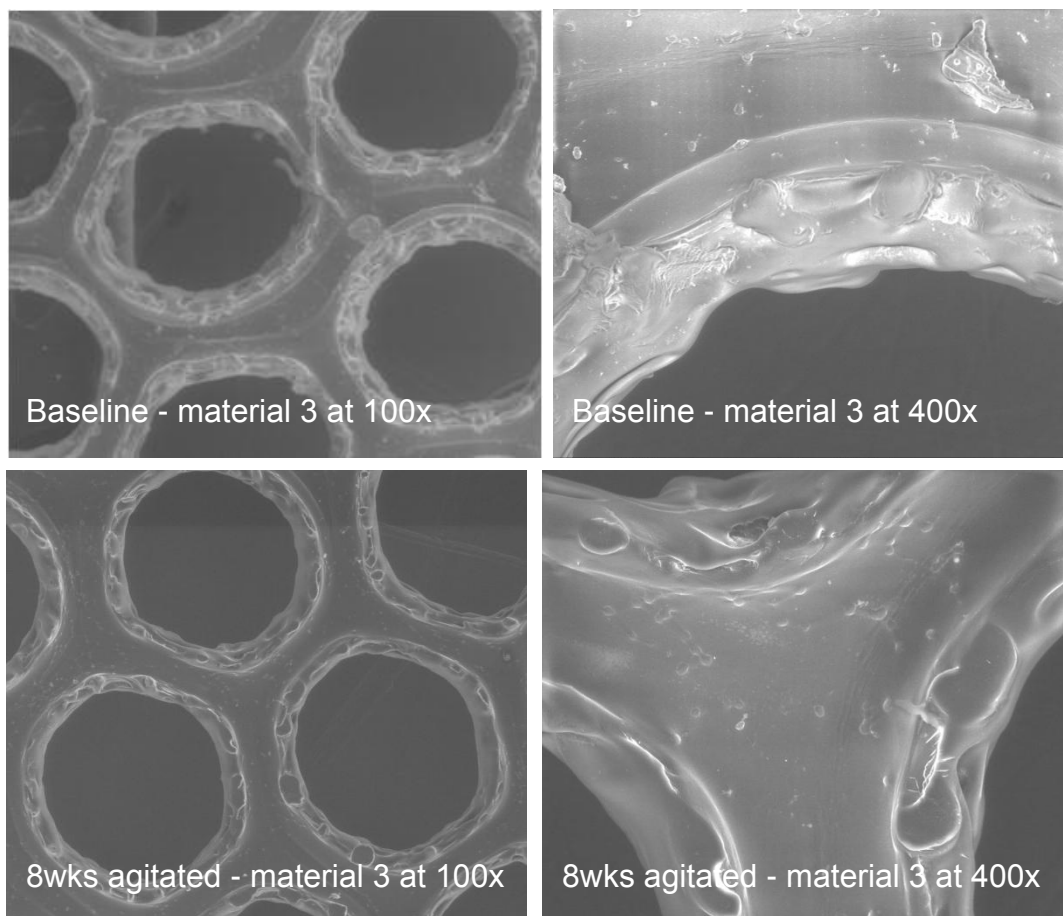


Figure 16: Baseline and 8 weeks agitated SEM images for PLLA film material 3 magnified 100 and 400 times

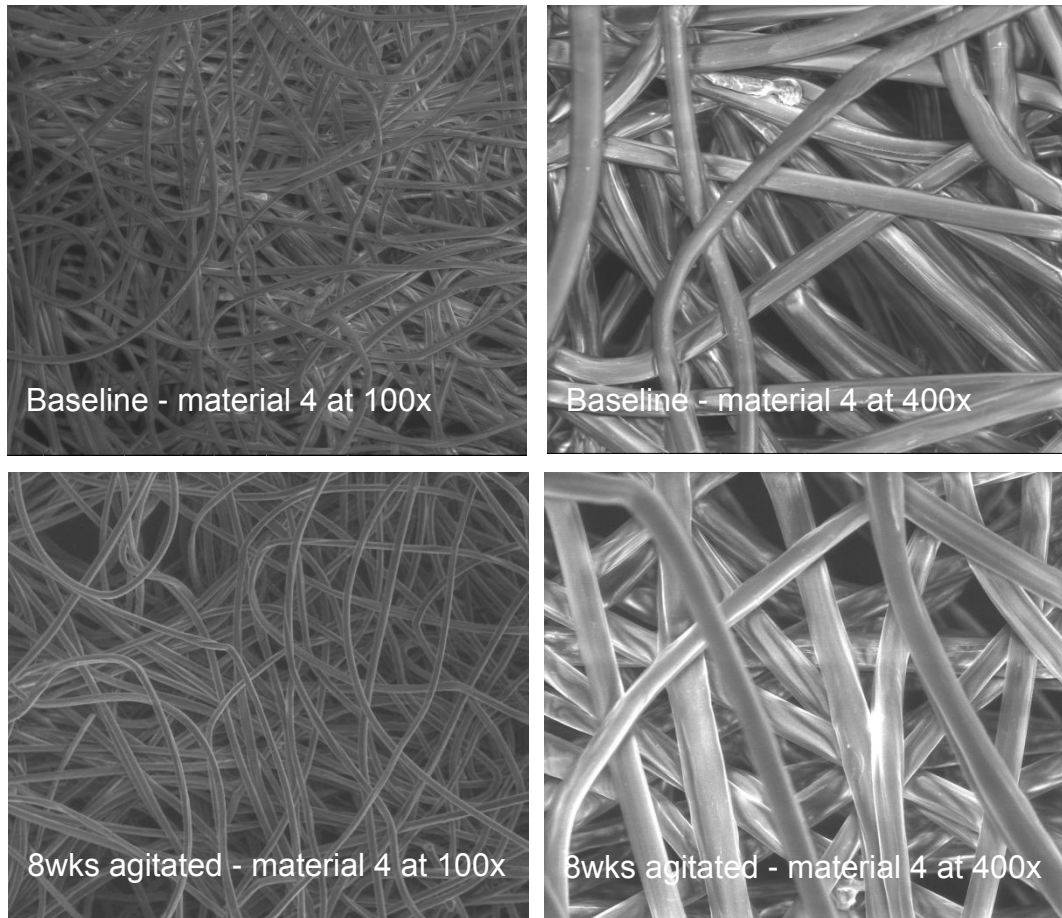


Figure 17: Baseline and 8 weeks agitated SEM images for PLLA BIOFELT material 4 magnified 100 and 400 times

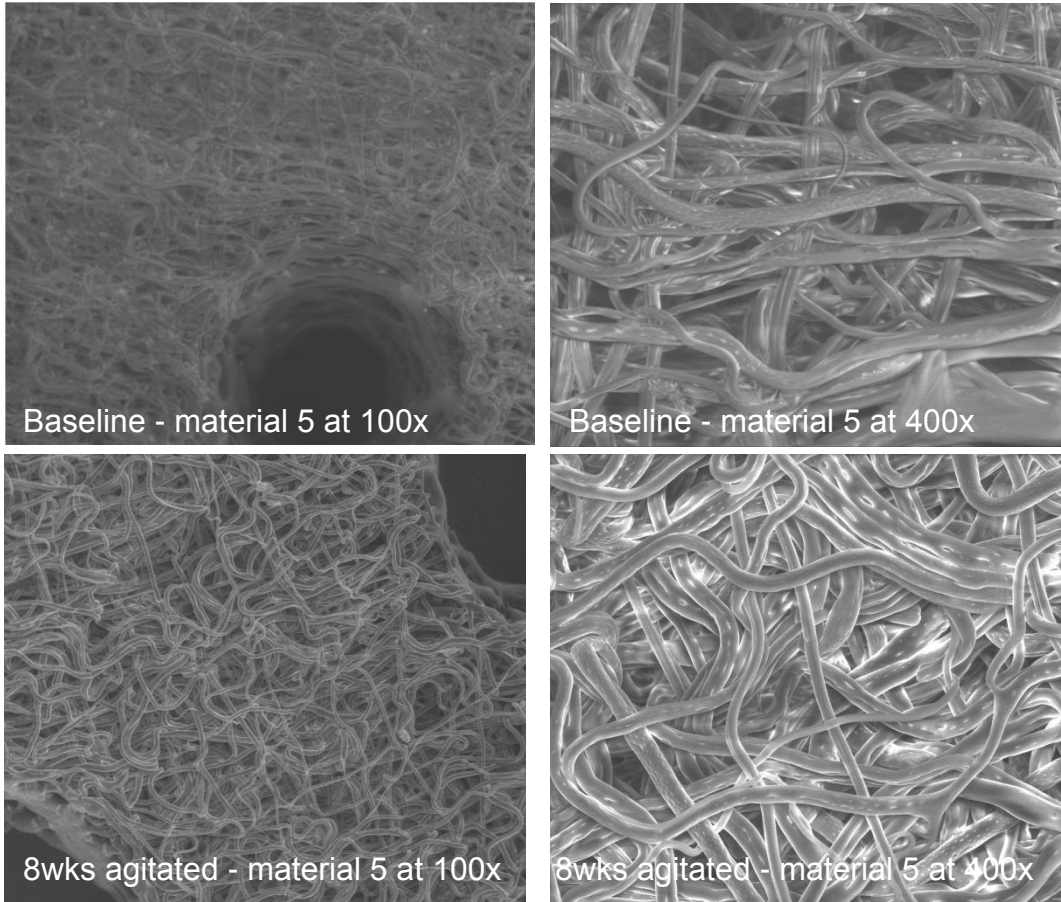


Figure 18: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 5 magnified 100 and 400 times

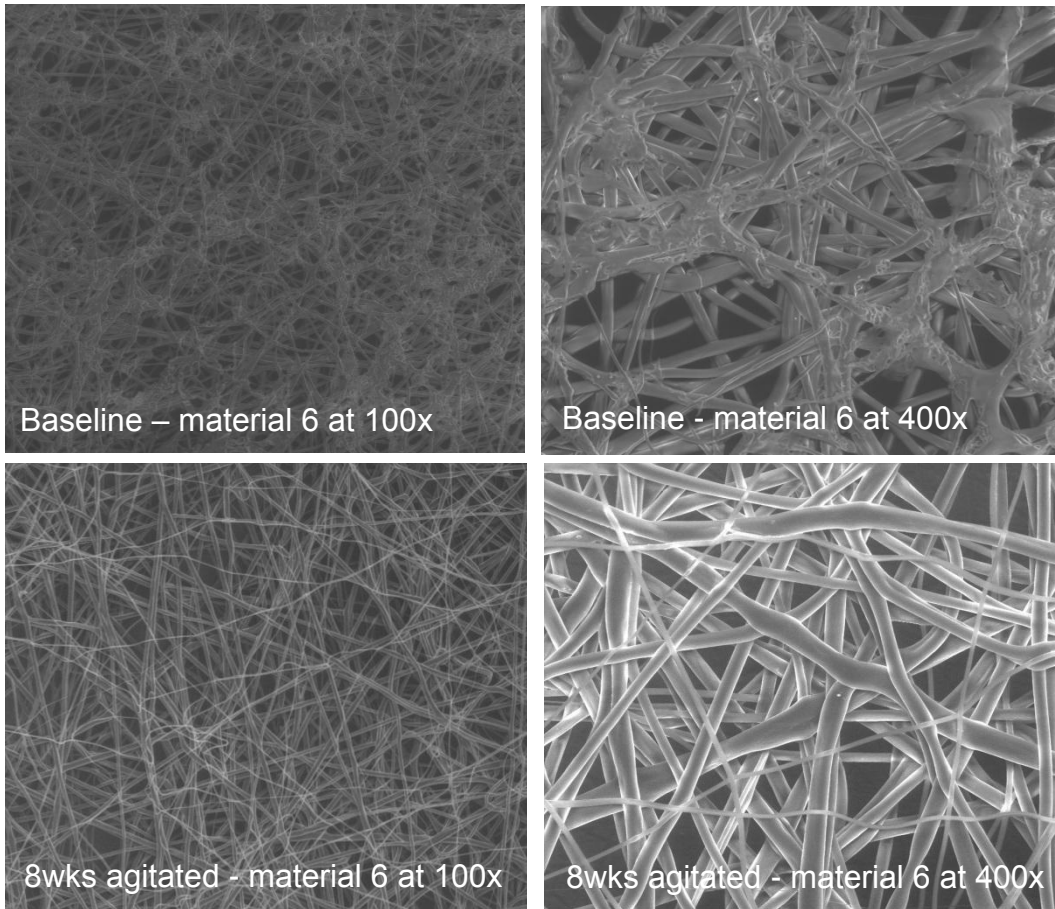


Figure 19: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 6 magnified 100 and 400 times

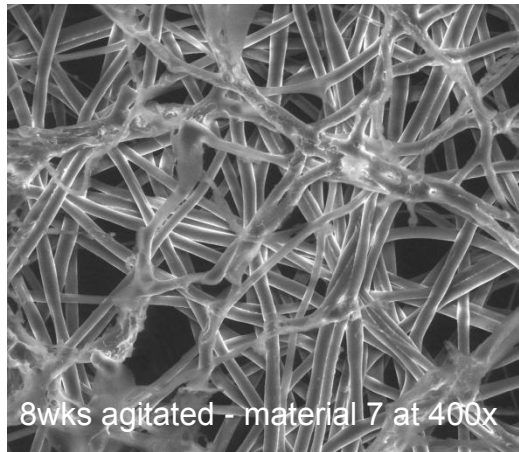
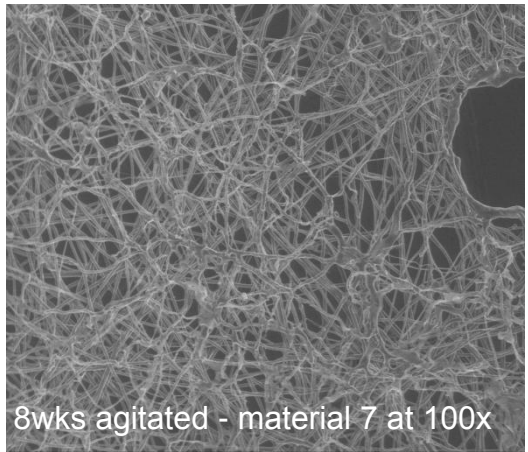
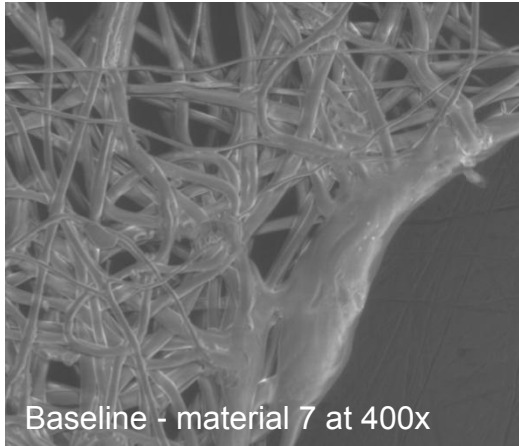
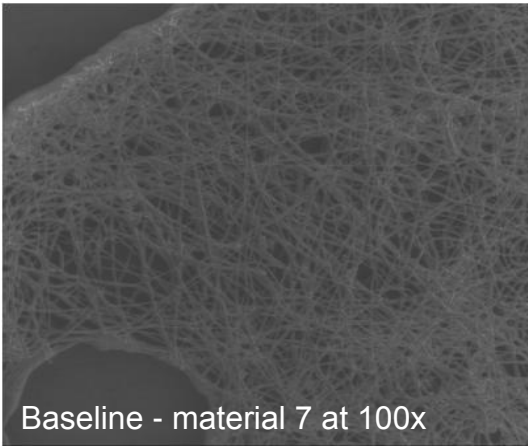


Figure 20: Baseline and 8 weeks agitated SEM images for PLLA/PCL electrospun material 7 magnified 100 and 400 times

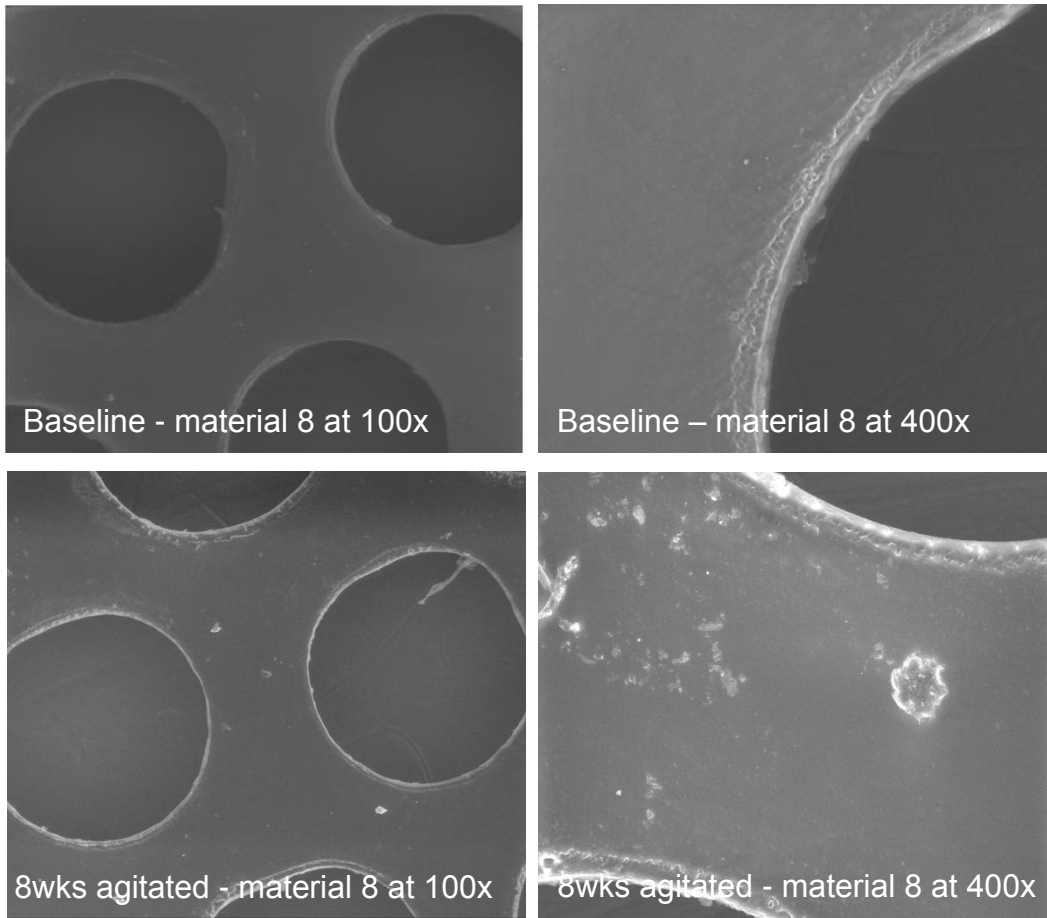


Figure 21: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 8 magnified 100 and 400 times

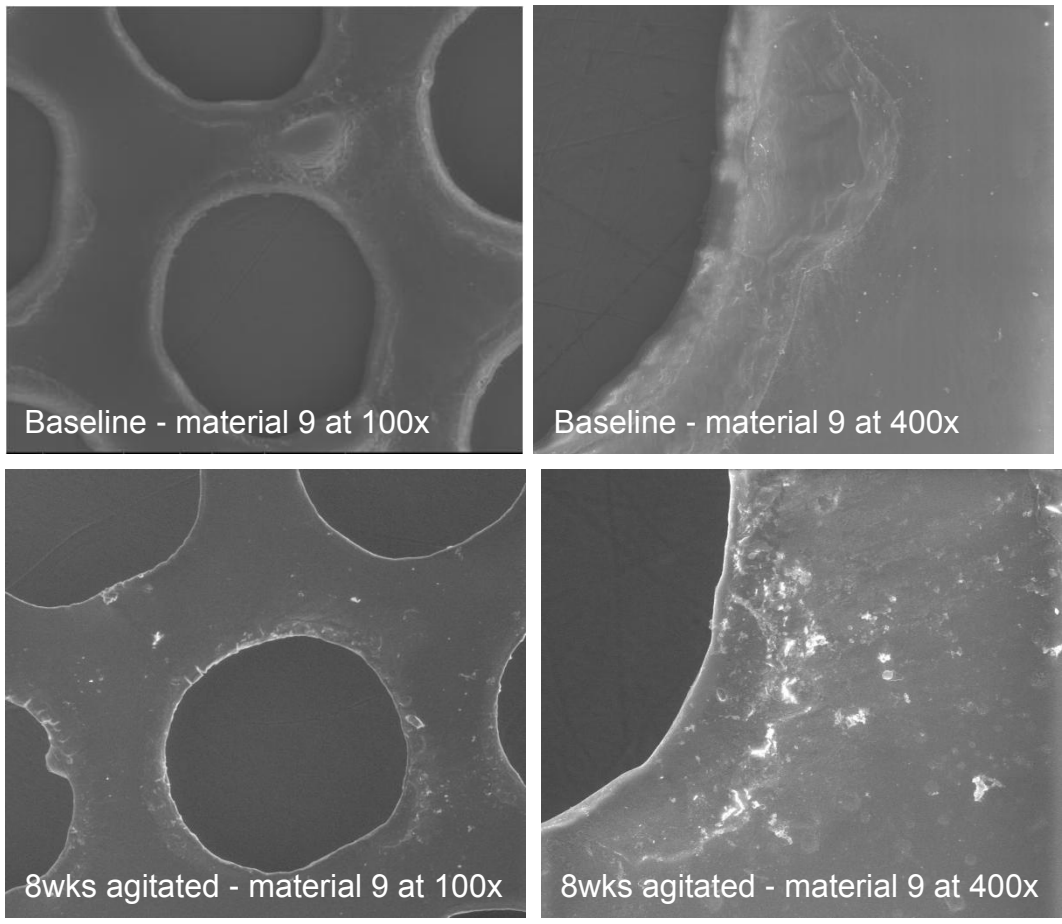


Figure 22: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 9 magnified 100 and 400 times

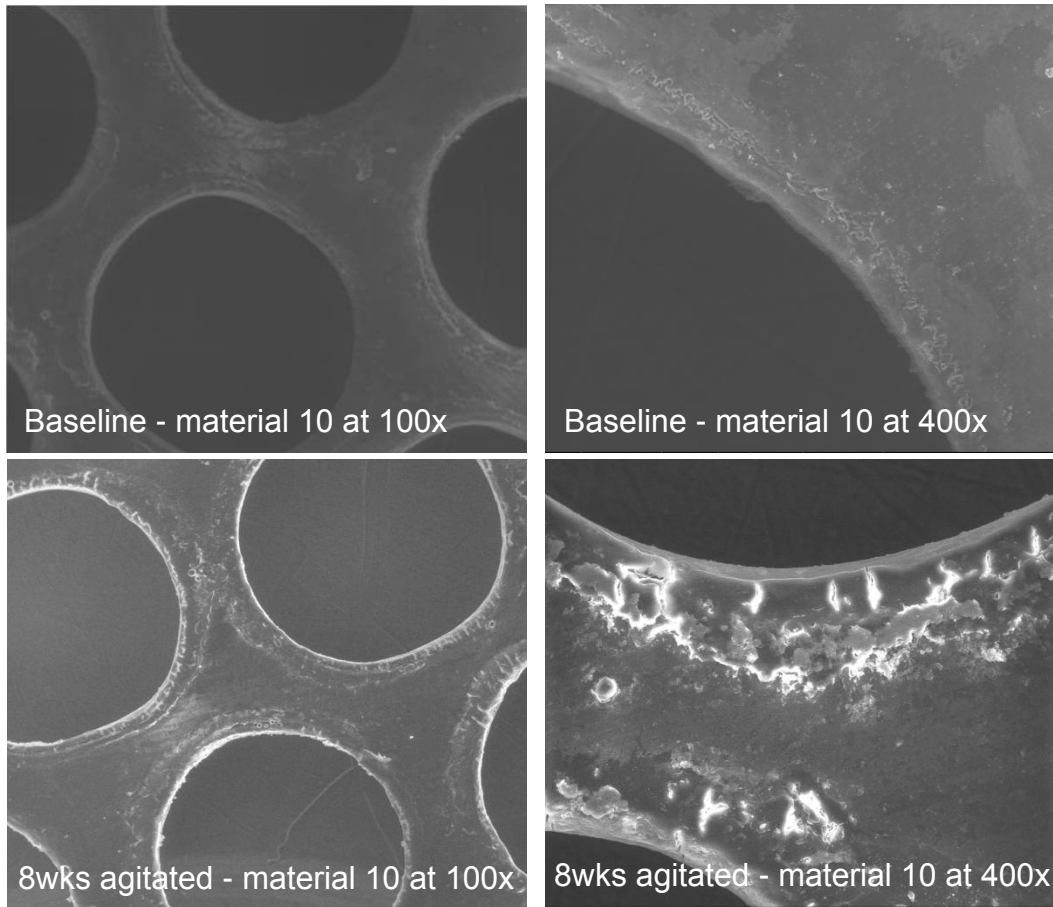


Figure 23: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 10 magnified 100 and 400 times

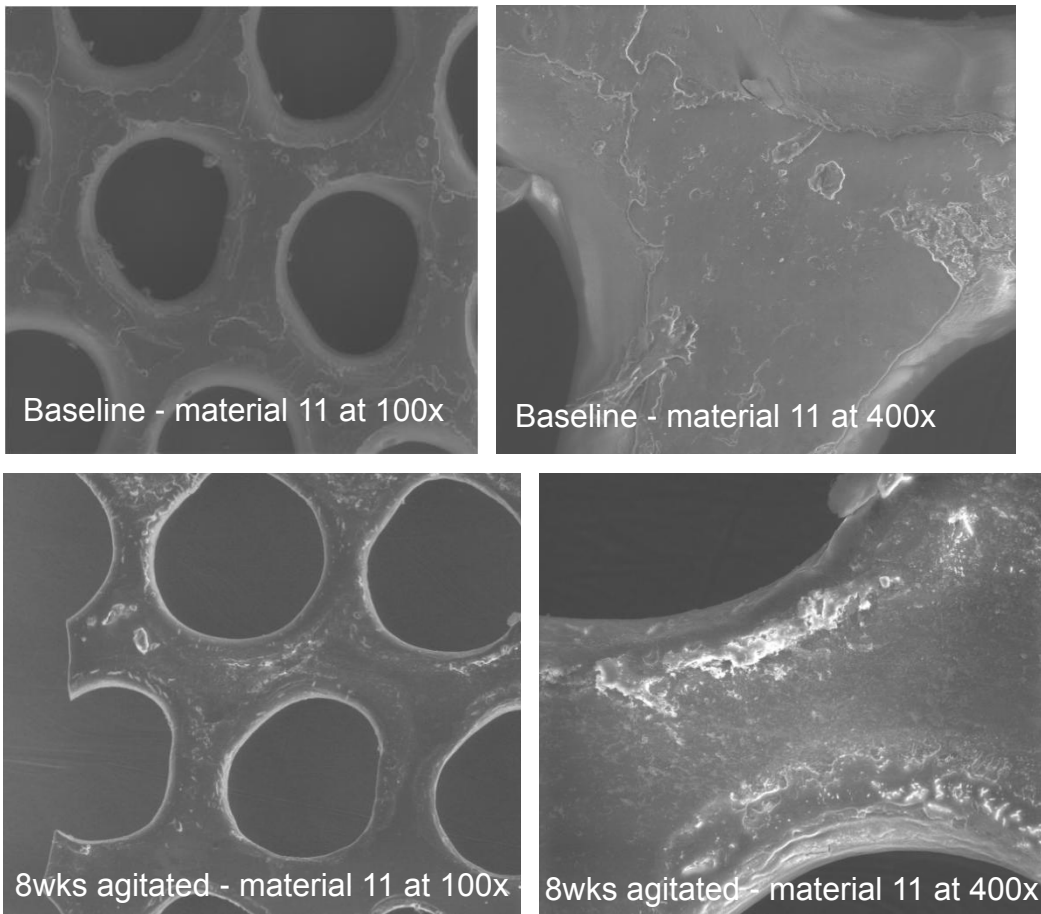


Figure 24: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 11 magnified 100 and 400 times

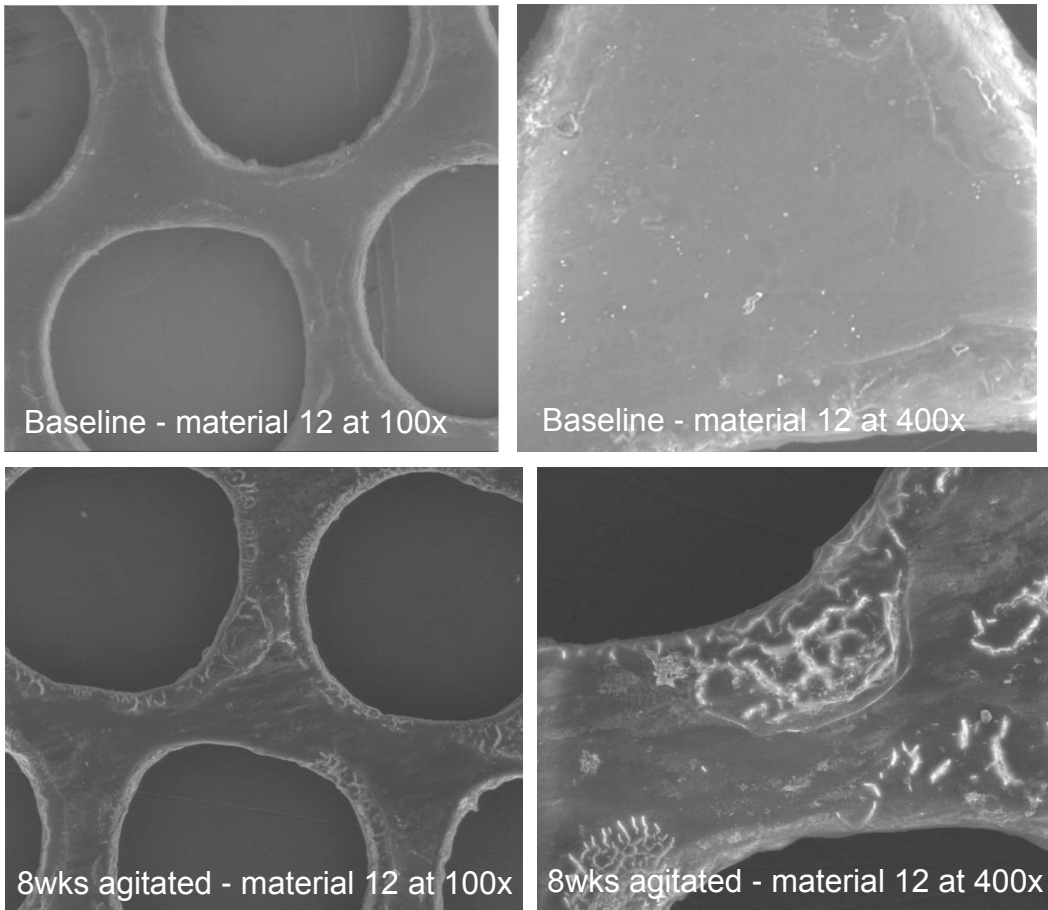


Figure 25: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 12 magnified 100 and 400 times

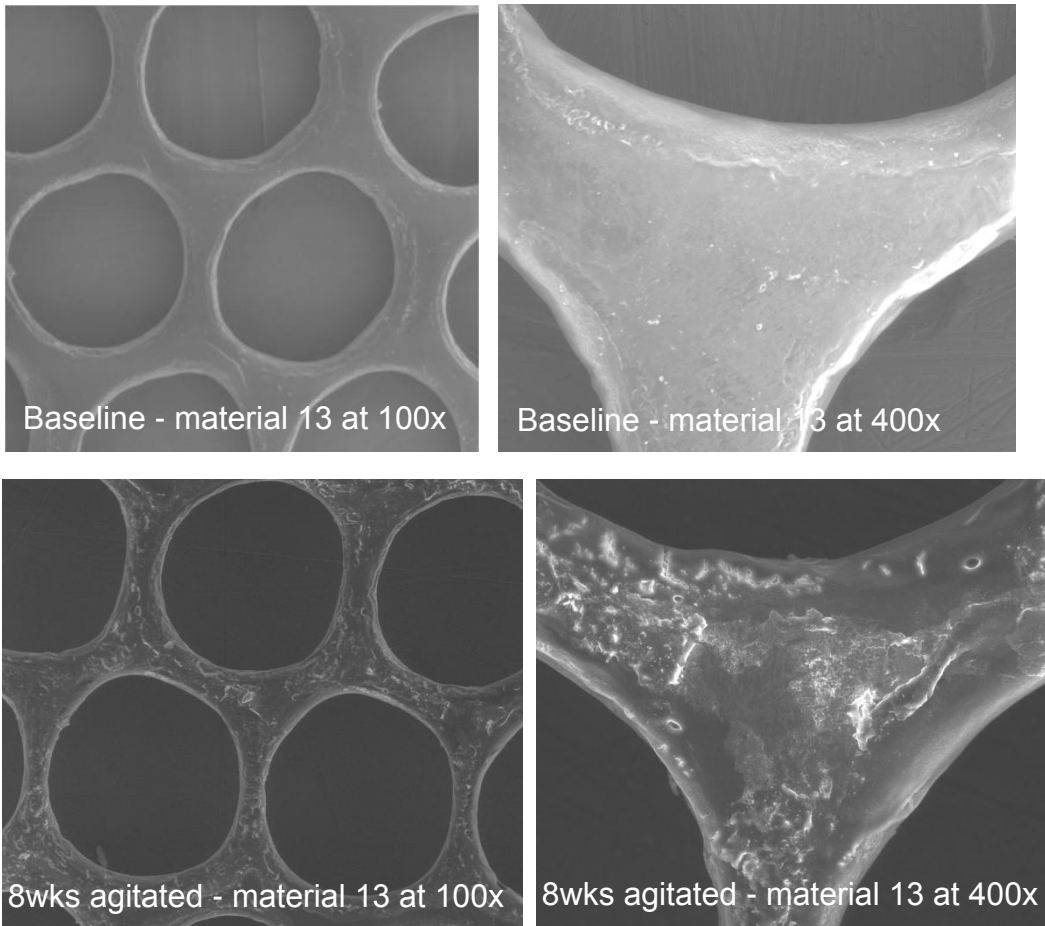


Figure 26: Baseline and 8 weeks agitated SEM images for PLLA/PCL film material 13 magnified 100 and 400 times

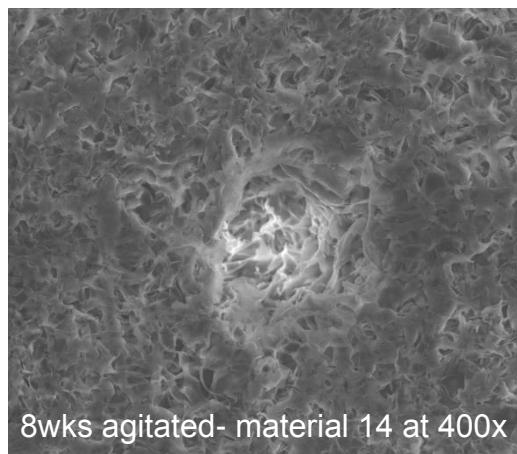
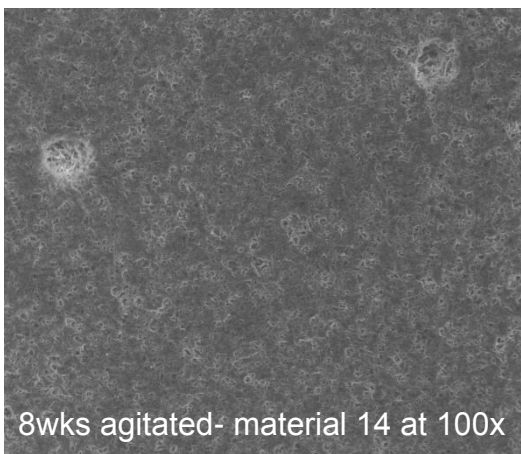
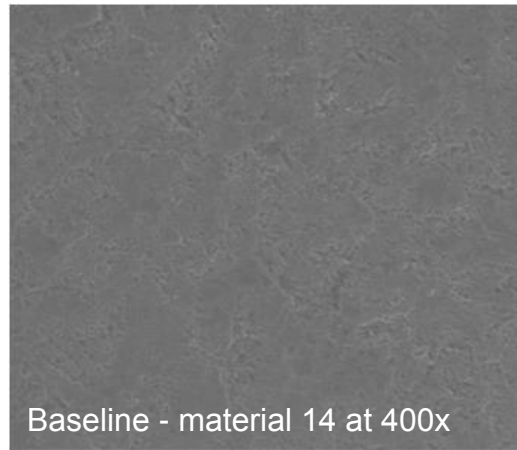
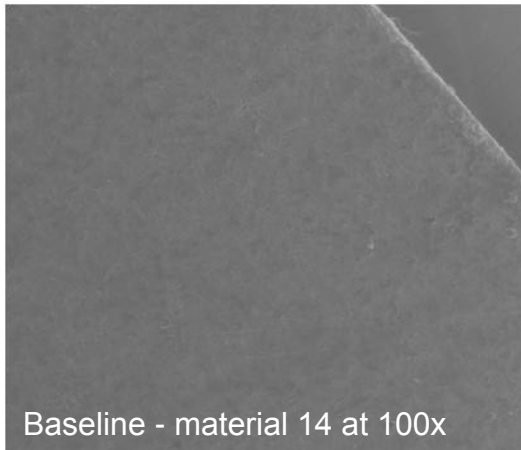


Figure 27: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 14 magnified 100 and 400 times

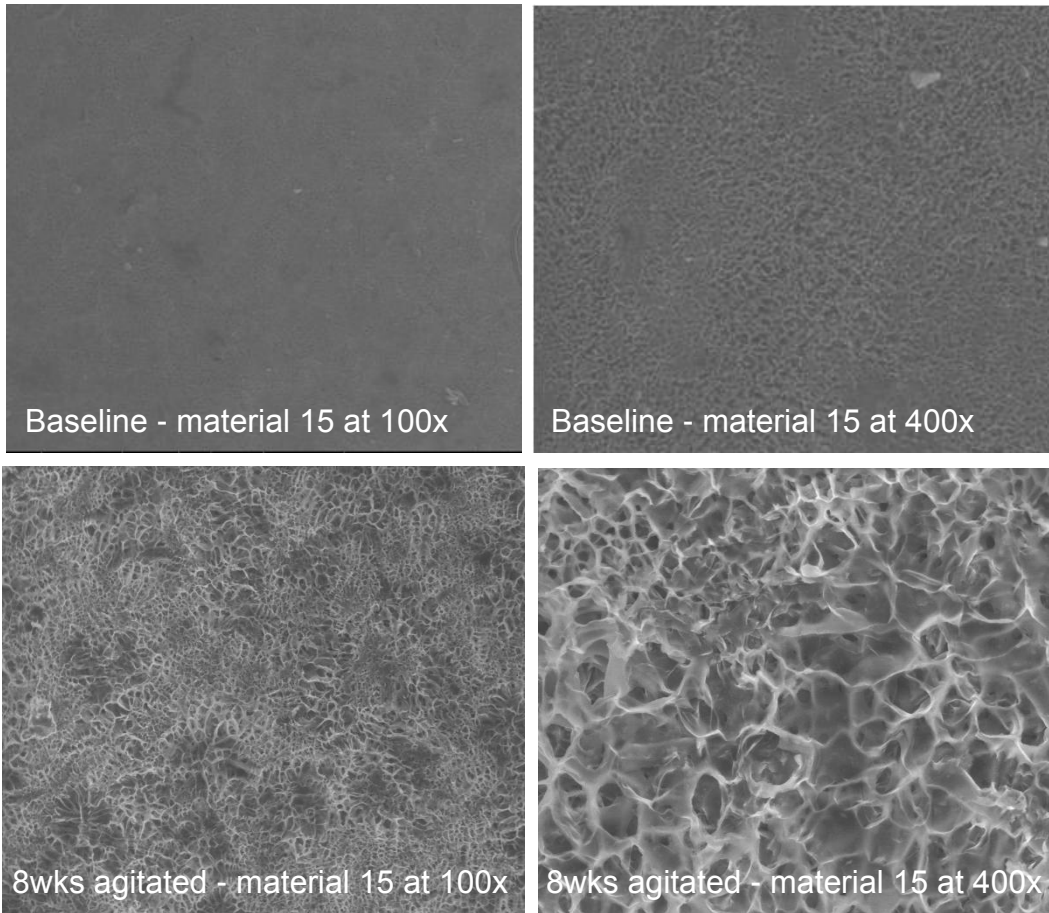


Figure 28: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 15 magnified 100 and 400 times

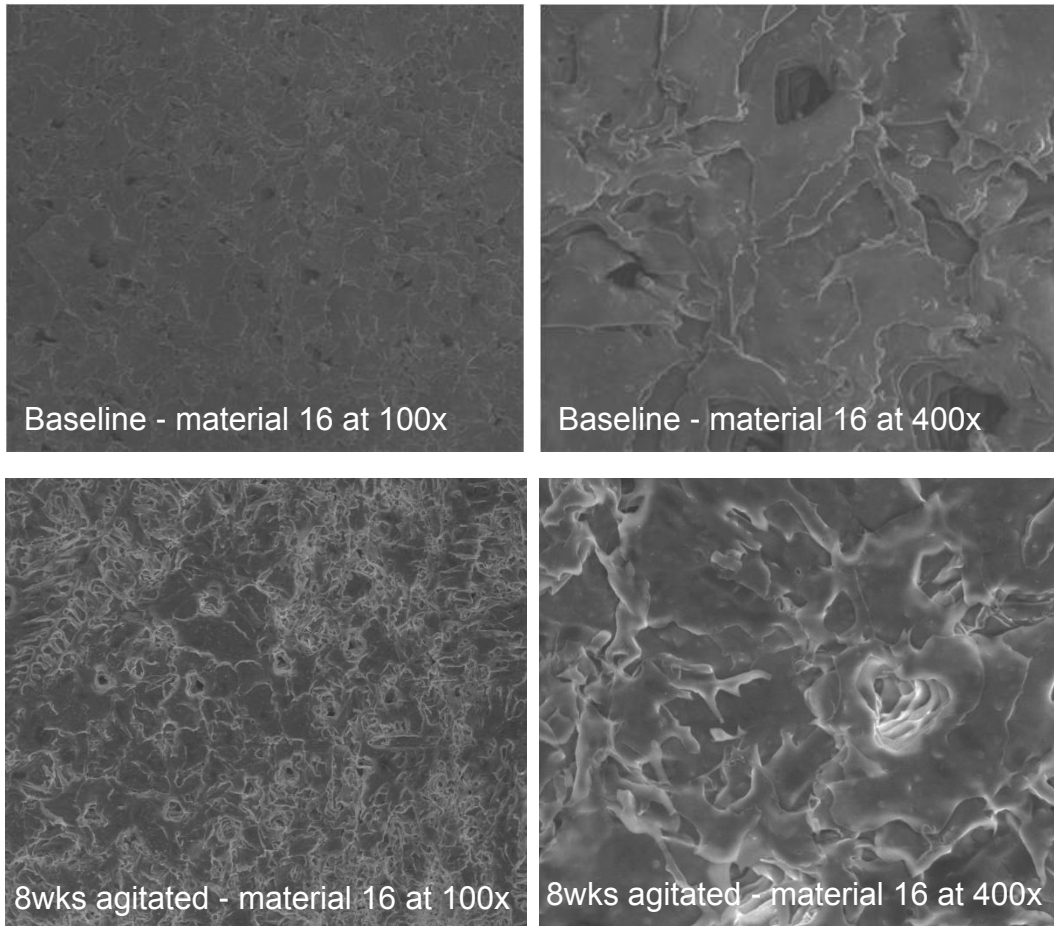


Figure 29: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 16 magnified 100 and 400 times

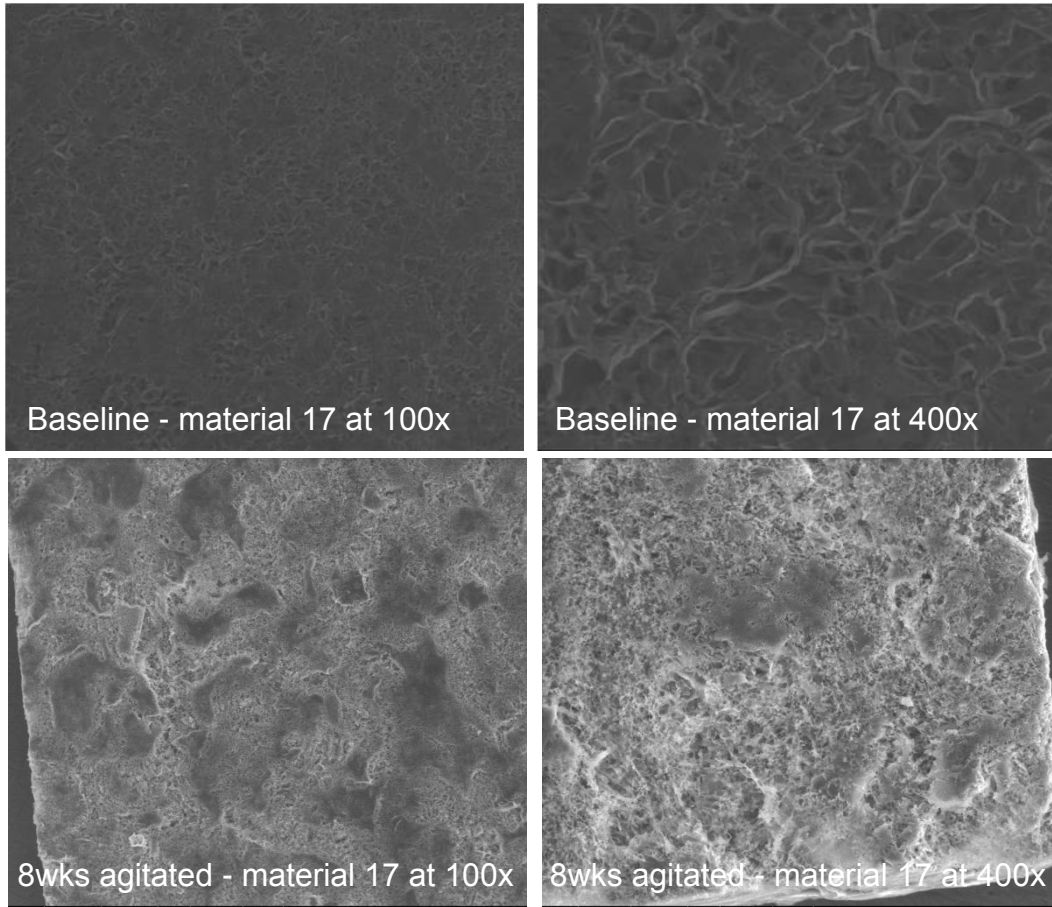


Figure 30: Baseline and 8 weeks agitated SEM images for PLLA/PCL sponge material 17 magnified 100 and 400 times

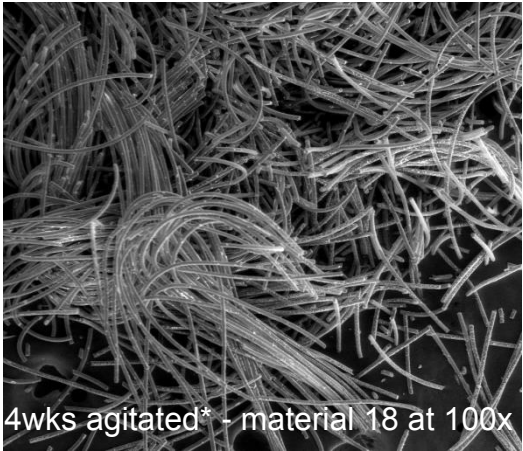
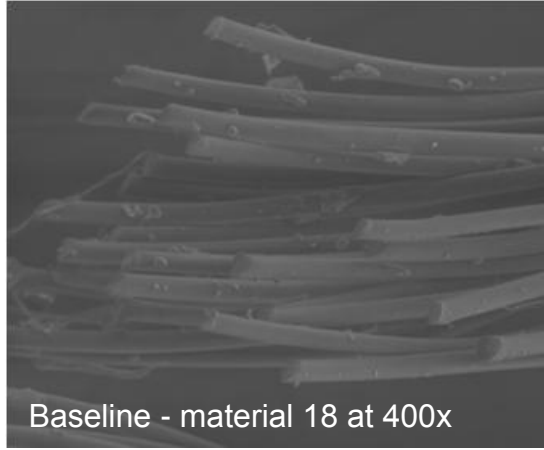
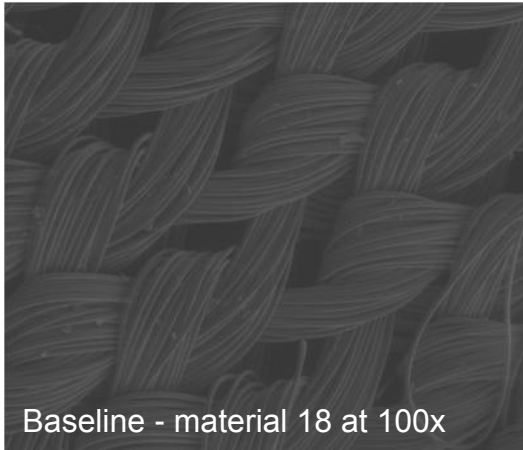


Figure 31: Baseline and 4 weeks agitated SEM images for PGA knit material 18 magnified 100 and 400 times

Gel Permeation Chromatography (GPC) Results and Analysis

Table 9: The average molecular weights of material samples (average of 3 samples) at baseline

Average Molecular Weight at Baseline (Daltons)			
Material	Average	Material	Average
1-BL	488452	10-BL	186032
2-BL	478550	11-BL	187238
3-BL	441904	12-BL	303532
4-BL	137012	13-BL	176727
5-BL	212149	14-BL	185358
6-BL	225717	15-BL	182455
7-BL	222509	16-BL	159383
8-BL	191354	17-BL	185225
9-BL	327606		

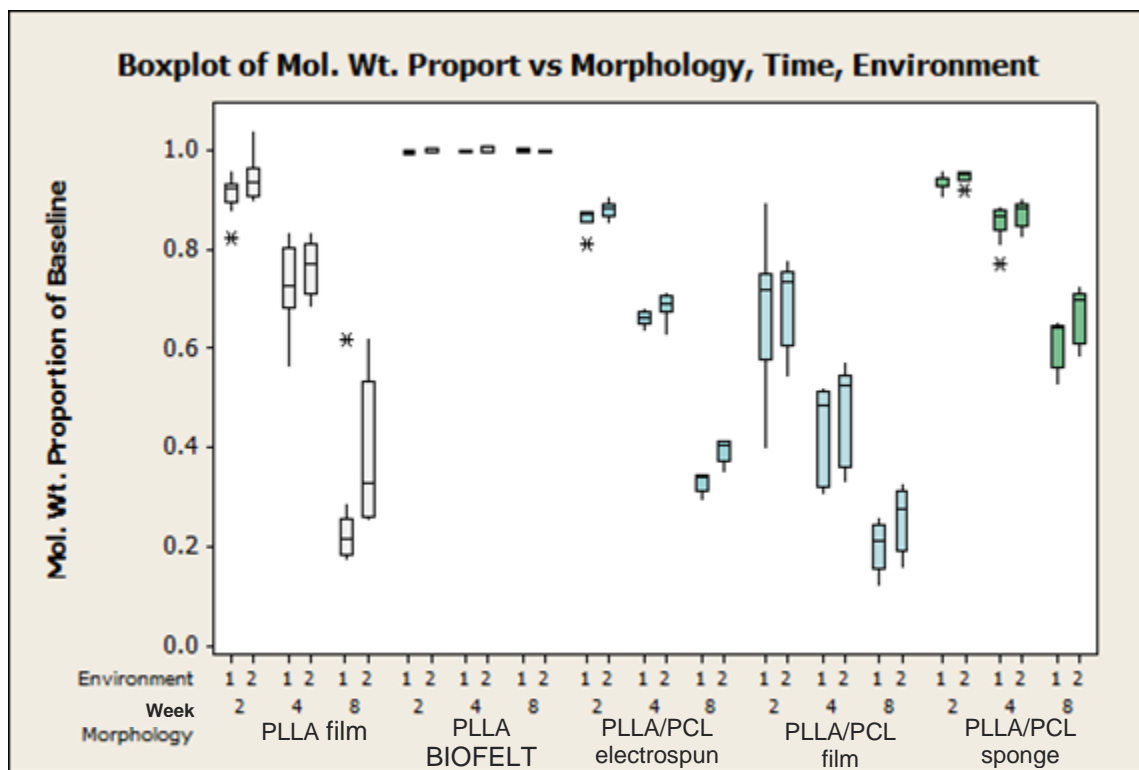


Figure 32: Boxplot of the proportion of the molecular weight at baseline by morphology, degradation time and degradation environment

GPC Data Analysis

There are several main effects as well as several interactions found in the proportional molecular weight data (see Appendix II for molecular weight Minitab ANOVA output). An initial three-way (Material x Degradation Time x Degradation Environment, $n = 3$) analysis of variance (ANOVA) demonstrated that all main effects and interactions were statistically significant ($p < 0.01$). The significance of the factor of material is expected as there were two separate chemical composition groups included in this ANOVA. The significance of the factor of time suggests that the molecular weight of the materials changed over time. Referencing Figure 13 it is clear that for all materials except material 4 there was a decrease in molecular weight, or degradation, at the time periods measured in this study (0-8 weeks). Also, it appears the degradation environment also generated significantly different measures of molecular weight which suggests possible differences in degradation in a stagnant vs. agitated environment. The significance of the interactions suggests that the molecular weights of each material changed differently with time and that the degradation environment affected the change in molecular weight of different materials with different magnitudes.

To investigate how each material's individual morphology (felt, electrospun, film, sponge and porosity/density) affected degradation a second set of three-way ANOVAs ($n = 3$) were performed for each chemical composition group (the PLLAs and the PLLA/PCLs). Interaction plots of the two data sets supported including all main effects and two factor interactions. Again, all main effects and interactions were significant ($p < 0.01$) with three exceptions. For all materials (both ANOVAs) the material-with-environment interaction was not significant and the environment-with-time interaction of materials 1 through 4 was also not significant. This would suggest that the material-environment interaction only occurs between the two material composition groups (PLLAs vs. PLLA/PCLs) and not within them. The significance of the factor of material for both data sets now demonstrates that the individual properties of the 17 materials has a statistically significant effect on molecular weight changes and the interaction of material

and degradation time also support a statistically significant difference in rate of change of molecular weight for the different materials. Once again, the degradation environment factor was also statistically significant suggesting differences in molecular weight related to the degradation environment.

Tensile Testing: Peak Strain Results and Analysis

Table 10: Values of the average peak strain for each of the materials at baseline (Mpa)

Average Peak Strain at Baseline (Mpa)			
Material	Average	Material	Average
1-BL	11.99	10-BL	677.48
2-BL	21.18	11-BL	548.69
3-BL	17.70	12-BL	644.91
4-BL	49.43	13-BL	476.69
5-BL	291.16	14-BL	32.97
6-BL	436.90	15-BL	71.72
7-BL	361.85	16-BL	80.83
8-BL	698.65	17-BL	24.85
9-BL	453.73	18-BL	452.22

For the peak strain analysis the values for material 18 are removed due to the fact that the material completely unraveled, making tensile testing impossible, after only two weeks.

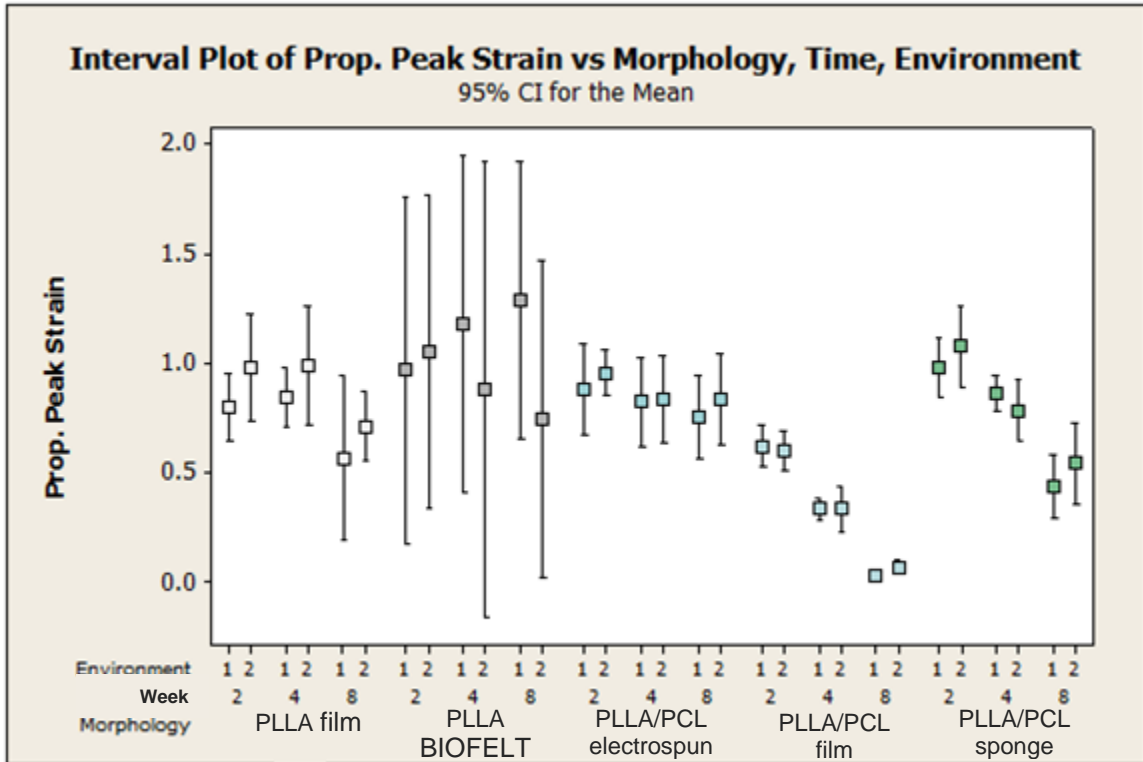


Figure 33: Interval plot of the peak strain proportion of baseline peak strain

Peak Strain Analysis

For statistical analysis of the peak strain data, a complete data set with replicates of 3 had to be produced from the mean and standard deviation for each material at each time point as a complete raw data set was not available.

An interaction plot of the proportion of peak strain from baseline by material, degradation time and degradation environment demonstrated that main effects and two factor interactions were present so these were all included in a 3-way ANOVA ($n = 3$) of the proportional strain (see Appendix III for proportional peak strain Minitab output). Only the main effect of the degradation environment and the interaction of degradation time and environment were not significant. This is interesting in that it suggests that although the molecular weight change was statistically different depending on the environment the difference was not great enough to generate a statistically significant change in the mechanical property of peak strain for these materials at the time points measured.

Further ANOVAs ($n = 3$) performed on the two chemical composition groups confirm that the effect of degradation environment was not significant for either set of materials. In addition, the effect of degradation time was not statistically significant for the PLLA group suggesting that the degradation of these materials was not sufficient to affect the peak strain behavior of these materials. This is not the case for the PLLA/PCL group where both main effects of material and degradation time as well as the material-time interaction were all significant ($p < 0.01$) indicating differences between materials, differences with increasing degradation time and different rates of behavior change over time for the different PLLA/PCL materials.

To further understand the degradation over time of individual materials one-way ANOVAs for each individual material (excluding material 18) by time were performed. As the factor of degradation environment was not significant, the data for each material in each environment were combined ($n=6$). PLLA materials 3 and 4 as well as

PLLA/PCL materials 5, 6, 7 and 17 were not significantly different ($p > 0.01$) up to the last point measured, 8 week degradation period. This would suggest that would indicate that degradation over 8 weeks did not significantly affect the strain behavior of these materials.

Table 11: Results of one-way ANOVAs of proportional peak strain by degradation time (Note: x means the difference between Tukey 95% confidence intervals included zero)

Results of One-way ANOVAs of Proportional Peak Strain by Degradation Time				
Material	p-value	2 weeks vs. 4 weeks	2 weeks vs. 8 weeks	4 weeks vs. 8 weeks
1	0.002			x
2	0.001	x		
3	0.107	x	x	x
4	0.995	x	x	x
5	0.969	x	x	x
6	0.151	x	x	x
7	0.038	x		x
8	0.000			
9	0.000			
10	0.000			
11	0.000			
12	0.000			
13	0.000			x
14	0.000			
15	0.000			
16	0.000	x		
17	0.021	x		x

Tensile Testing: Peak Stress Results and Analysis

Table 12: Values of the average peak stress at baseline (Mpa)

Average Peak Stress at Baseline (Mpa)			
Material	Average	Material	Average
1-BL	15.34	10-BL	6.87
2-BL	24.00	11-BL	6.61
3-BL	13.74	12-BL	5.86
4-BL	0.10	13-BL	3.24
5-BL	1.79	14-BL	2.23
6-BL	6.25	15-BL	1.43
7-BL	3.37	16-BL	3.34
8-BL	9.50	17-BL	0.70
9-BL	6.00	18-BL	18.67

For the peak stress analysis the values for material 18 are removed due to the fact that the material became completely unraveled, making tensile testing impossible, after only two weeks.

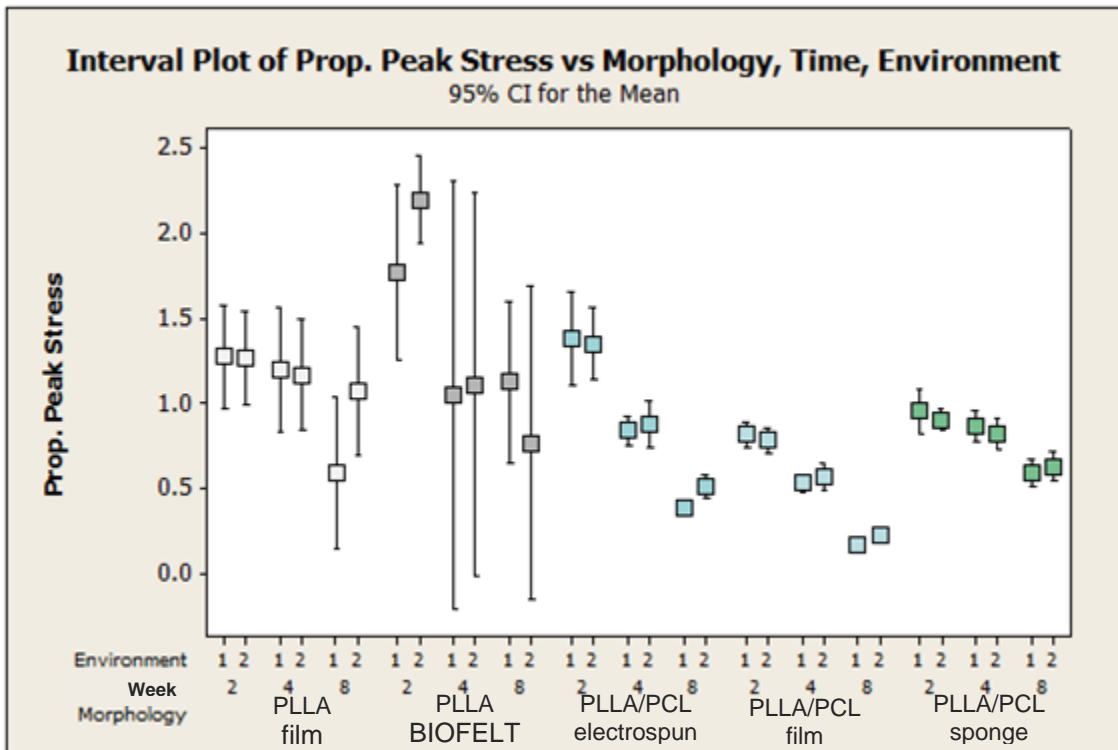


Figure 34: Interval plot of the peak stress proportion of baseline peak stress

Peak Stress Analysis

As with strain, for analysis of the peak stress data a set of 3 replicates had to be produced from mean and standard deviation data for each material at each time point as the complete raw data were not available.

An interaction plot of the proportion of peak stress from baseline by material, degradation time and degradation environment demonstrated that main effects and two factor interactions were present so these were all included in a three-way ANOVA (n=3) of the proportional stress (see Appendix IV for proportional peak stress Minitab output). Only the main effect of the degradation environment and the two interactions involving degradation environment were not significant. This finding again supports the notion that the molecular weight change was not sufficient to generate a statistically significant change in the behavior of the materials measured by peak stress at the time points measured.

Further ANOVAs (n=3) performed on the two chemical composition groups confirm that the effect of degradation environment was not significant for either set of materials. Contrary to strain measurements, the effect of time and material (as well as the interaction between the two) was significant ($p < 0.01$) for the PLLA group suggesting that the change in molecular weight of these materials was sufficient to affect the stress behavior of the materials. The PLLA/PCL group was the same indicating differences between materials, differences with increasing degradation time and different rates of behavior change over time for all the different materials measured.

Table 13: Results of one-way ANOVAs of proportional peak stress by degradation time (Note: x means the difference between 95% confidence intervals included zero)

Results of One-way ANOVAs of Proportional Peak Stress by Degradation Time				
Material	p-value	2 weeks vs. 4 weeks	2 weeks vs. 8 weeks	4 weeks vs. 8 weeks
1	0.121	x	x	x
2	0.000			
3	0.017	x		
4	0.000			
5	0.000	x		
6	0.000			
7	0.000			
8	0.000			
9	0.000			
10	0.000			
11	0.000			
12	0.000			
13	0.000			
14	0.000	x		
15	0.003	x		x
16	0.000	x		
17	0.006	x		

One-way ANOVAs for each individual material (excluding material 18) by time (n=6) were performed for proportional peak stress. PLLA materials 1 and 3 were not significantly different ($p > 0.01$) over the 8 week degradation period. This would suggest that any changes in molecular weight did not significantly affect the stress behavior of these materials. When comparing 95% confidence intervals (CIs) for each material by degradation time several materials, PLLAs 1 and 3 as well as PLLA/PCLs 5, 14, 15, 16 and 17 did not significantly change between 2 weeks and 4 weeks. This would suggest that the rate of change related to degradation time for these materials was slightly slower for these materials until the 4 week time point.

Conclusions and Recommendations

Initial visual inspection, SEM images and DSC data did not single out any particular material for recommendation as a bioresorbable material for congenital heart defect repair. However, the PGA material (material 18) would not be recommended as an implantable material based on these tests as it did not maintain any kind of structural integrity beyond a 2 week period. It might be a candidate if it can be processed in such a way that it does not unravel but then it would need to be retested as no tests could be performed on this material after 4 weeks and no strength testing was possible after 2 weeks. The slight degradation related changes visible in the SEM images of materials 4 through 17 suggest that these materials may be appropriate for bioresorbable materials as the changes noted may promote tissue ingrowth. Further in vitro and in vivo testing would be necessary to confirm this. With the exception of the PGA material and possibly the PLLA/PCL sponge material 14, no major damage or large pieces of material broke off of the material samples. Major damage such as this would rule out a material as large pieces degrading into the blood stream could potentially create an embolism or stroke.

GPC measurements of all the materials suggest that there was molecular weight change with degradation. The magnitude of degradation depended on the individual material properties, degradation time and degradation environment for all the materials except possibly material 4, the PLLA BIOFELT, which maintained molecular weights very near baseline values. The magnitude changes were statistically significant for all the main factors of material, degradation time and degradation environment ($p < 0.01$). The change in molecular weight within the first 2 to 8 weeks would theoretically be beneficial, potentially allowing for tissue ingrowth.

PLLA film material 3 was the only material tested here to maintain its mechanical properties over the 8 week degradation time period as measured by peak strain and peak stress. PLLA film material 1 maintained its stress behavior and PLLA BIOFELT material 4 and PLLA/PCL materials 5 through 7 all maintained their strain behavior over

the 8 weeks degradation time. The materials that maintained at least one of these mechanical measurements over time would be the strongest candidates for further testing. The other materials may still be acceptable depending on how much of a difference (statistically significant or not) is acceptable. For instance, PLLA BIOFELT material 4 maintained its strain performance but not its stress performance and the baseline value for the material was on the order of human cardiac tissue tensile strength (Chen et al., 2008) so a significant decrease from that value does not make it a strong candidate for congenital heart defect repair. The other materials tested here all had peak stress values at least an order of magnitude greater than human cardiac tissue but less than collagen so these materials may still not be appropriate (Chen et al., 2008). In addition, there were only enough samples to test these materials in one direction so if any materials are anisotropic further testing would be required.

Although the results from these tests are inconclusive regarding specific recommendations for bioresorbable materials for congenital heart defect repair there are a few important results. First, the exact morphology and chemical composition of the material does have a significant effect on the degradation rate as measured by the change in molecular weight over time. This suggests that if an ideal degradation rate is determined for the cardiac environment the PLLA and/or PLLA/PCL materials investigated here could be manufactured in a specific morphology to closely match the desired degradation profile. In addition, there were a few materials that maintained their stress and strain behavior for several weeks which suggest it is possible for these materials to undergo some degradation while maintaining mechanical properties for up to 8 weeks when it is thought that endothelialization and tissue ingrowth should be in progress. Further testing would be required to see for how long the mechanical strength is maintained. It is recommended that further testing include testing materials at longer degradation time points; to at least 24 weeks up to 2 years if possible, to get a profile for degradation behavior out to the time point where it is thought that complete endothelialization and tissue ingrowth are solidly underway and/or complete (Sigler & Jux, 2007). In addition, testing of the mechanical properties of atrial septal tissue itself

would be very helpful in determining if the mechanical properties of these materials are appropriate and what are the minimal properties required for atrial septal wall and device integrity to be maintained during the ingrowth process.

As there were significant differences in molecular weight, but not peak stress and strain measurements, the inclusion of an agitated or dynamic degradation environment is still recommended. Especially for degradation in a dynamic environment of a beating heart one would ideally test degradation beyond agitation at 80 RPM and include testing with solution flowing across the material and/or the materials being agitated against another surface that would simulate the placement of these materials flush with cardiac septal tissue to get a more accurate agitated environment measure. In addition, this testing was completed in a relatively constant (minor fluctuations in pH), inert solution of phosphate buffered saline. If bioresorbable materials are to be successfully implanted and resorbed, testing that includes representations of immune system response and other blood factors should be completed in vitro first. For instance, one could add the enzymes present in the blood stream, such as lipase, to see if active components significantly change the degradation characteristics of these materials. One should also determine the tissue ingrowth feasibility further by attempting to seed the materials with cells to determine if tissue ingrowth is likely to be successful.

References

- AMPLATZER TM septal occluder. (2012). Retrieved 1/21, 2013, from <http://www.sjmprofessional.com/Products/US/structural-heart-therapy/amplatzer-septal-occluder.aspx>
- Boucek, M. B. (2005). Patent foramen ovale closure: Role of the pediatric cardiologist. *Cardiology Clinics*, 23(1), 35-45. doi:10.1016/j.ccl.2004.10.011
- Chen, Q., Bismarck, A., Hansen, U., Junaid, S., Tran, M. Q., Harding, S. E., . . . Boccaccini, A. R. (2008). Characterisation of a soft elastomer poly (glycerol sebacate) designed to match the mechanical properties of myocardial tissue. *Biomaterials*, 29(1), 47-57.
- Chessa, M., Butera, G., Frigiola, A., & Carminati, M. (2004). Endothelialization of ASD devices for transcatheter closure: Possibility or reality? *International Journal of Cardiology*, 97(3), 563-564.
- Davis, D., Gregson, J., Willeit, P., Stephan, B., Al-Shahi Salman, R., & Brayne, C. (2013). Patent foramen ovale, ischemic stroke and migraine: Systematic review and stratified meta-analysis of association studies. *Neuroepidemiology*, 40(1), 56-67. doi:10.1159/000341924

Davidson, C., & Bonow, R. (2008). Chapter 20 - Cardiac Catheterization. In P. Libby, R. Bonow, D. Mann, D. Zipes & E. Braunwald (Eds.), *Braunwald's Heart Disease - A textbook of cardiovascular medicine* (9th Edition ed., pp. 394) Elsevier Saunders.

Fisher, D. F. (1995). The incidence of patent foramen ovale in 1,000 consecutive PatientsA contrast transesophageal echocardiography study. *Chest*, *107*(6), 1504.
doi:10.1378/chest.107.6.1504

GORE^(R) HELEX^(R) septal occluder

. (2012). Retrieved 1/21, 2013, from <http://www.goremedical.com/helex/>

Guchlerner, M., Kardos, P., Liss Koch, E., Franke, J., Wunderlich, N., Bertog, S., & Sievert, H. (2012). PFO and right-to-left shunting in patients with obstructive sleep apnea. *Journal of Clinical Sleep Medicine*, *8*(4), 375-380. doi:10.5664/jcsm.2026

Hara, H., Hara, R., Virmani, E., Ladich, S., Mackey Bojack, J., Titus, M., . . . Schwartz. (2005). Patent foramen ovale: Current pathology, pathophysiology, and clinical status. *Journal of the American College of Cardiology*, *46*(9), 1768-1776.
doi:10.1016/j.jacc.2005.08.038

Jux, C., Bertram, H., Wohlsein, P., Brüggemann, M., Wüboldt, P., Fink, C., . . . Hausdorf, G. (2002). Experimental ASD closure using autologous cell-seeded interventional closure devices. *Cardiovascular Research*, *53*(1), 181-191. doi:10.1016/S0008-6363(01)00442-4

Jux, C., Bertram, H., Wohlsein, P., Bruegmann, M., & Paul, T. (2006). Interventional atrial septal defect closure using a totally bioresorbable occluder matrix: Development and preclinical evaluation of the BioSTAR device. *Journal of the American College of Cardiology*, 48(1), 161-169. doi:10.1016/j.jacc.2006.02.057

Khessali, H., Mojadidi, M. K., Gevorgyan, R., Levinson, R., & Tobis, J. (2012). The effect of patent foramen ovale closure on visual aura without headache or typical aura with migraine headache. *JACC Cardiovascular Interventions*, 5(6), 682-687. doi:10.1016/j.jcin.2012.03.013

Kupferschmid, C., & Lang, D. (1983). The valve of the foramen ovale in interatrial right-to-left shunt: Echocardiographic cineangiographic and hemodynamic observations. *The American Journal of Cardiology*, 51(9), 1489-1494.

Kutty, S., Sengupta, P., & Khandheria, B. (2012). Patent foramen ovale: The known and the to be known. *Journal of the American College of Cardiology*, 59(19), 1665-1671. doi:10.1016/j.jacc.2011.09.085

Medical policy: Closure devices for patent foramen ovale and atrial septal defects (ASD). (2012). Retrieved 1/21, 2013, from https://www.premera.com/medicalpolicies/cmi_003467.htm

Mojadidi, M. K., Khessali, H., Gevorgyan, R., Levinson, R., & Tobis, J. (2012). Visual migraine aura with or without headache: Association with right to left shunt and

assessment following transcatheter closure. *Clinical Ophthalmology*, 6, 1099-1105. doi:10.2147/OPHTH.S30999

Mügge, A., Daniel, W. G., Angermann, C., Spes, C., Khandheria, B. K., Kronzon, I., . . . Engberding, R. (1995). Atrial septal aneurysm in adult patients a multicenter study using transthoracic and transesophageal echocardiography. *Circulation*, 91(11), 2785-2792.

Nagpal, S., Lerakis, S., Flueckiger, P., Halista, M., Willis, P., Block, P., . . . Babaliaros, V. (2013). Long-term outcomes after percutaneous patent foramen ovale closure. *The American Journal of the Medical Sciences*, doi:10.1097/MAJ.0b013e318276b071

Nir, A., Driscoll, D., & Edwards, W. (1994). Intrauterine closure of membranous ventricular septal defects: Mechanism of closure in two autopsy specimens. *Pediatric Cardiology*, 15(1), 33-37.

Patten, B., & Patten. (1931). The closure of the foramen ovale. *American Journal of Anatomy*, 48(1), 19-44. doi:10.1002/aja.1000480104

Percy, R. F., Conetta, D. A., Geiser, E. A., Bass, T. A., Conti, C. R., & Miller, A. B. (1985). Correlation of paradoxical atrial septal motion and an interatrial pressure gradient in severe tricuspid regurgitation. *The American Journal of Cardiology*, 55(11), 1431-1433.

Perloff, J. K. (1971). Therapeutics of nature--the invisible sutures of "spontaneous closure". *American Heart Journal*, 82(5), 581-585.

Proprietary ePTFE technology from Gore. (2012). Retrieved 1/21, 2013, from http://www.gore.com/en_xx/products/venting/packaging/eptfe_membrane.html

Rao, P. S., & Sissman, N. J. (1971). Spontaneous closure of physiologically advantageous ventricular septal defects. *Circulation*, 43(1), 83-90.

Rigatelli, G., Dell'avvocata, F., Cardaioli, P., Giordan, M., Braggion, G., Aggio, S., . . . Chinaglia, M. (2012). Improving migraine by means of primary transcatheter patent foramen ovale closure: Long-term follow-up. *American Journal of Cardiovascular Disease*, 2(2), 89-95.

Savelle, J. (2009). Characterization of Mechanical Properties and In-Vitro Degradation Kinetics of Polyester-based Biomaterials for Potential Patent Foramen Ovale Applications. Unpublished Manuscript. University of Minnesota. Department of Biomedical Engineering. Minneapolis, MN.

Sigler, M., & Jux, C. (2007). Biocompatibility of septal defect closure devices. *Heart*, 93(4), 444-449.

The Cleveland Clinic Foundation. (3/30/2012). Treatments and procedures: How is a patent foramen ovale (PFO) closed using a catheter-based procedure? Retrieved 1/21, 2013, from

http://my.clevelandclinic.org/services/endovascular_patent_foramen_ovale_pfo_closure/hic_how_is_a_patient_foramen_ovale_pfo_closed_using_catheter_based_procedure.aspx

Zahn, E. M., Wilson, N., Cutright, W., & Latson, L. A. (2001). Development and testing of the helex septal occluder, a new expanded polytetrafluoroethylene atrial septal defect occlusion system. *Circulation*, 104(6), 711-716.

Appendices

Appendix I: DSC

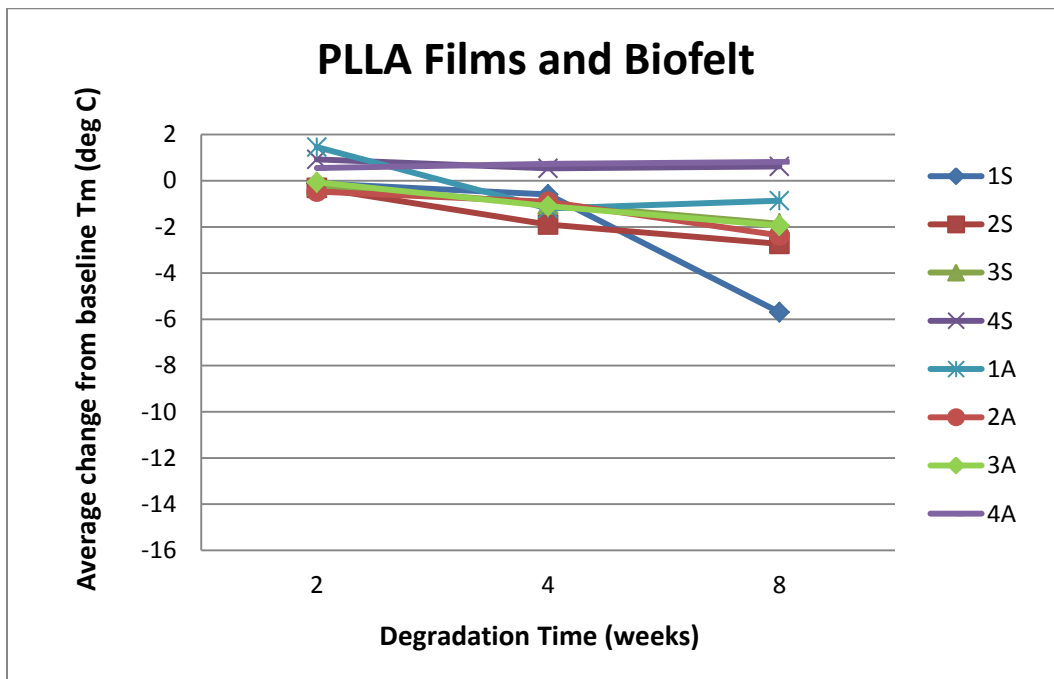


Figure 35: Average change from baseline Tm for PLLA films and biofelt

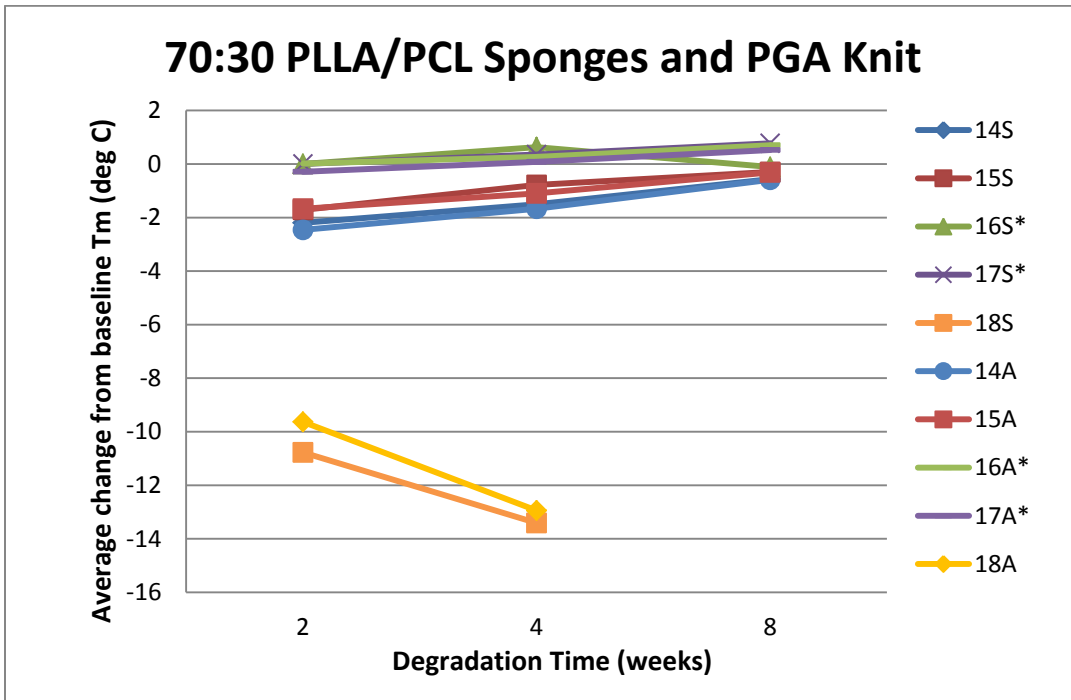


Figure 36: Average change from baseline Tm for 70:30 PLLA/PCL sponges and PGA knit
 Note: material 18 has no 8S or 8A data

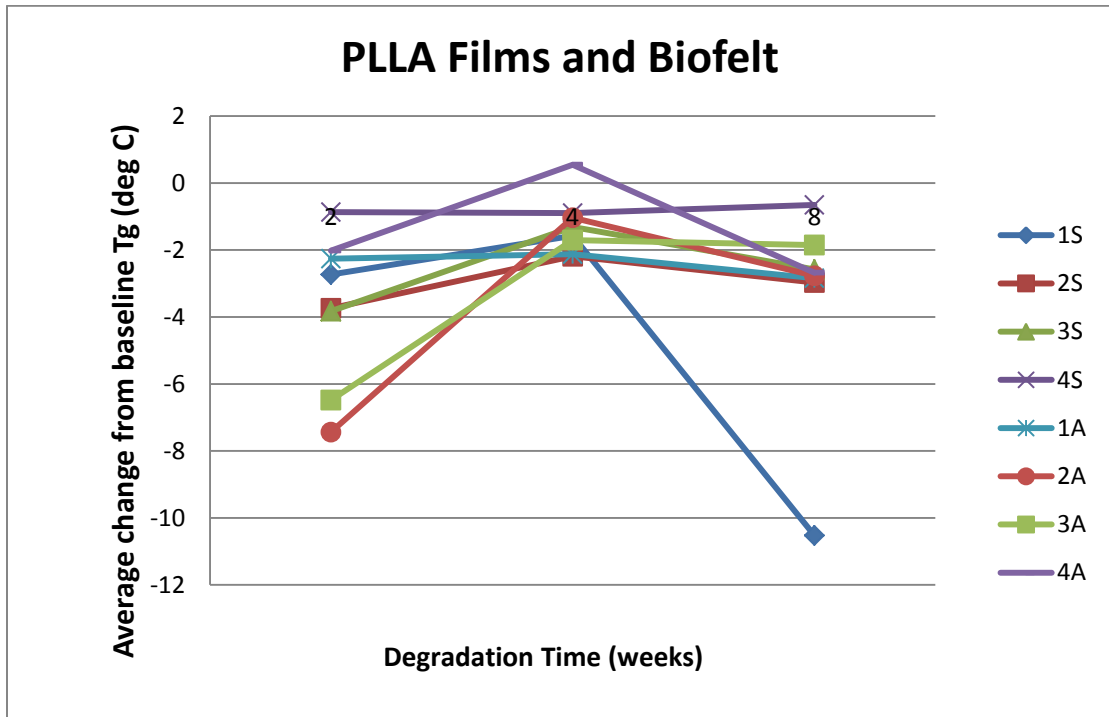


Figure 37: Average change from baseline Tg for PLLA films and Biofelt (Note: this is a different scale)

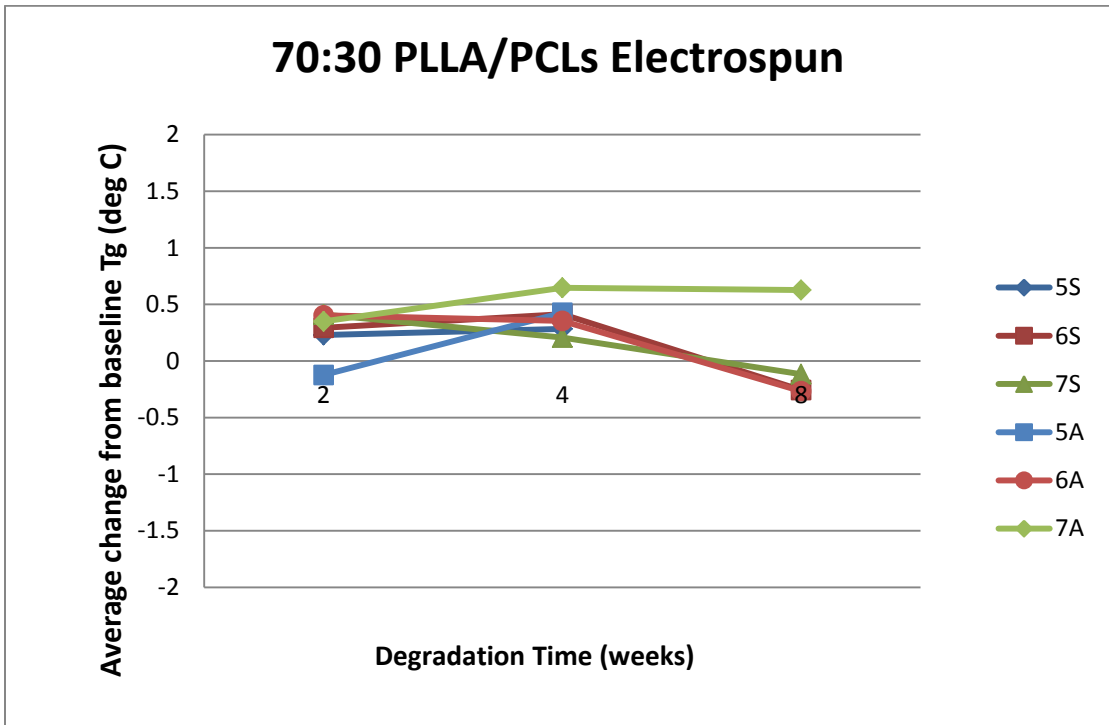


Figure 38: Average change from baseline Tg for electrospun 70:30 PLLA/PCL

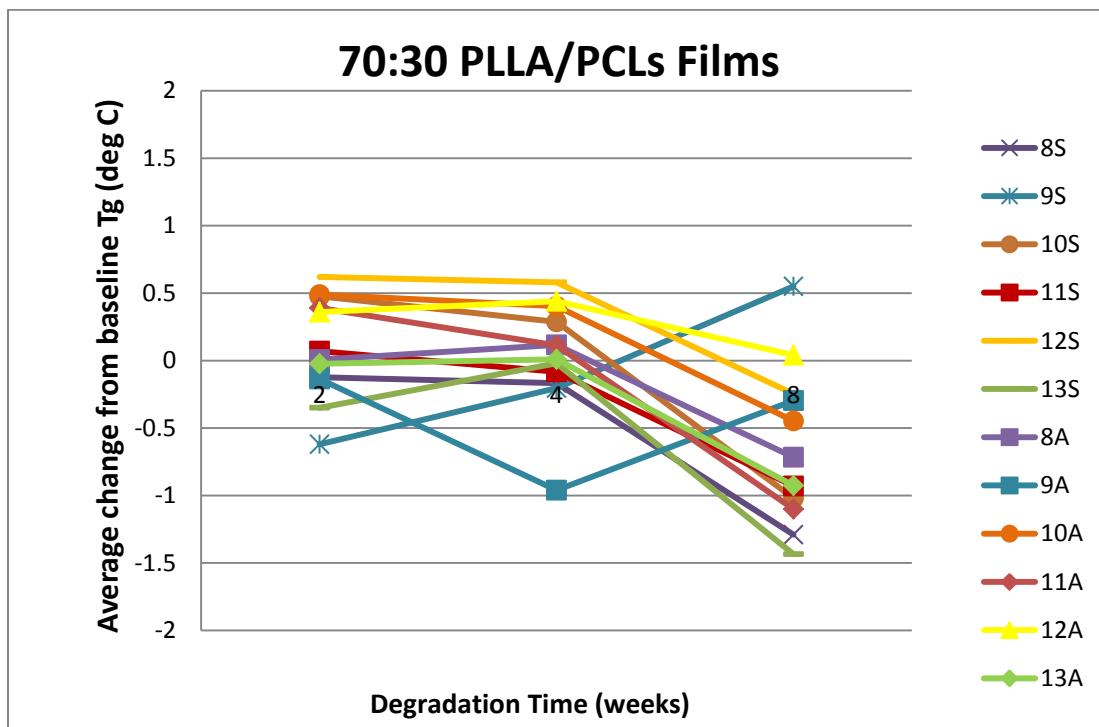


Figure 39: Average change from baseline Tg of 70:30 PLLA/PCL films

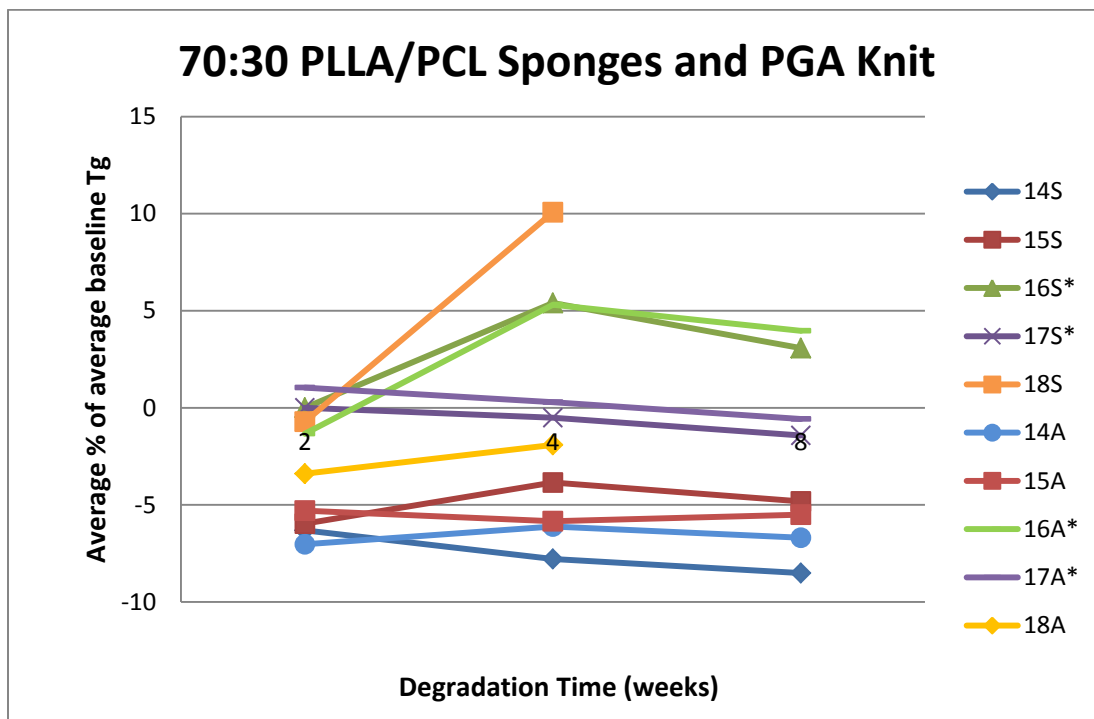


Figure 40: Average change from baseline Tg for 70:30 PLLA/PCL sponges and PGA knit (Note: this is a different scale)

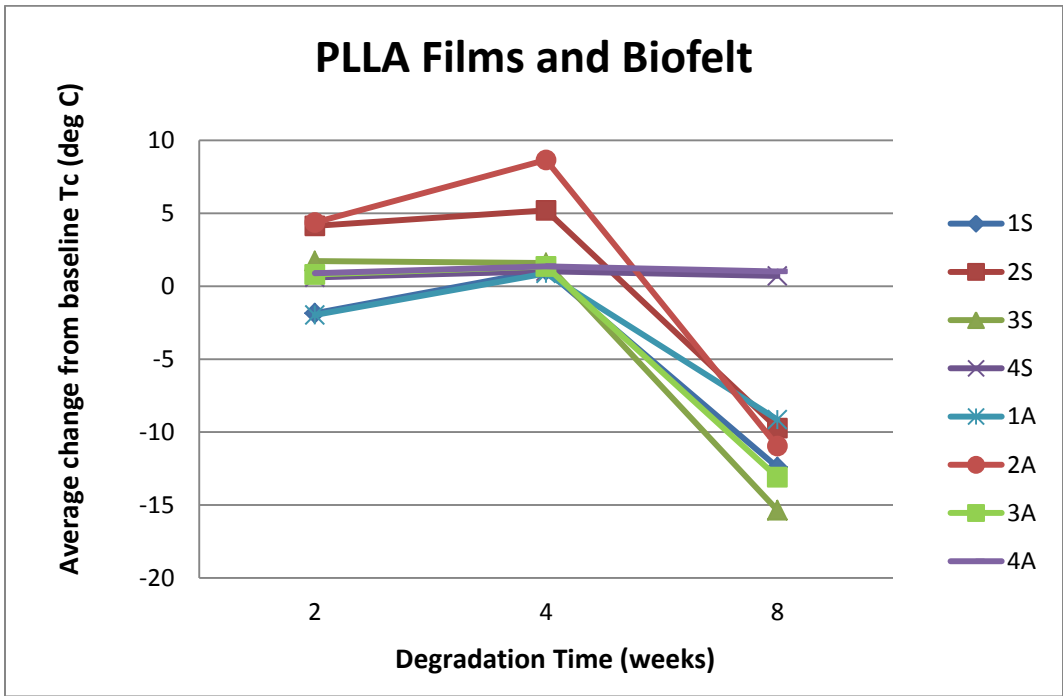


Figure 41: Average change from baseline Tc for PLLA films and Biofelt samples

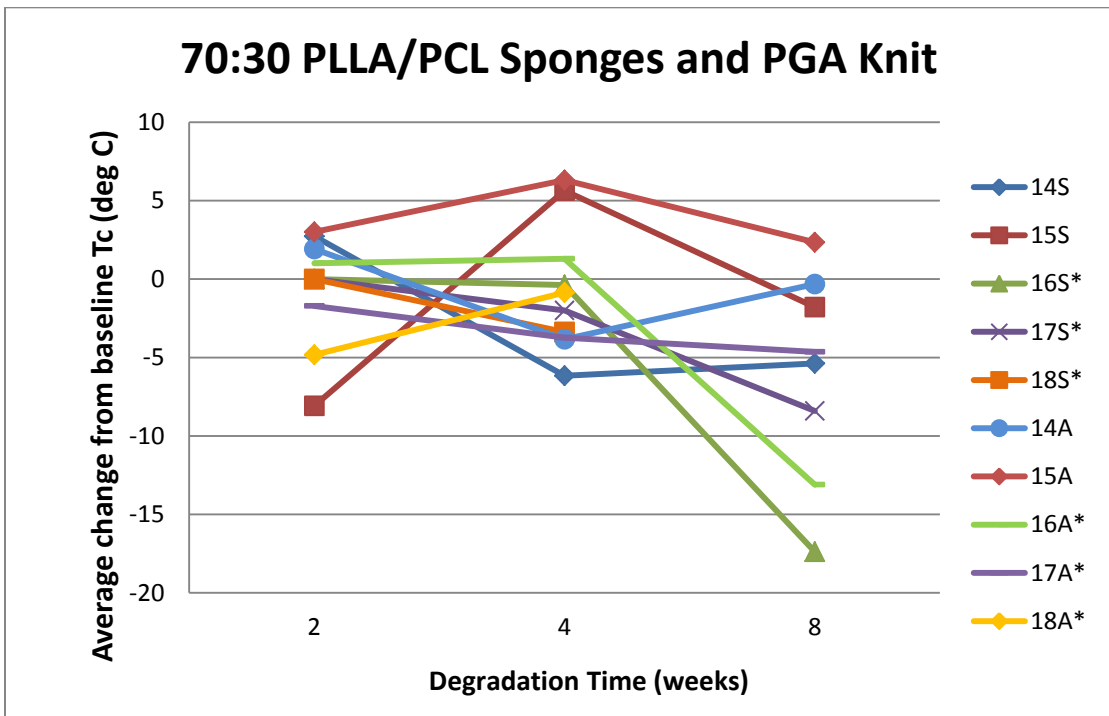


Figure 42: Average change from baseline Tc for 70:30 PLLA/PCL sponges and PGA knit samples

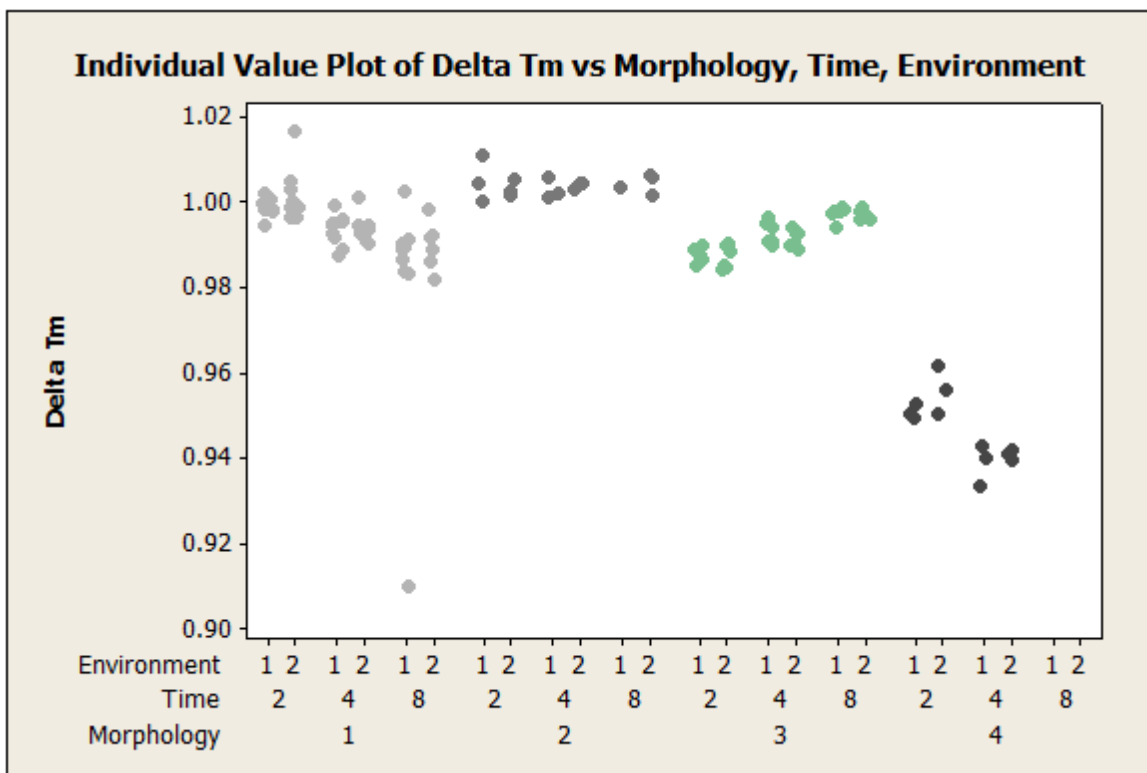


Figure 43: Individual value plot of the change in Tm by morphology, degradation time and degradation environment

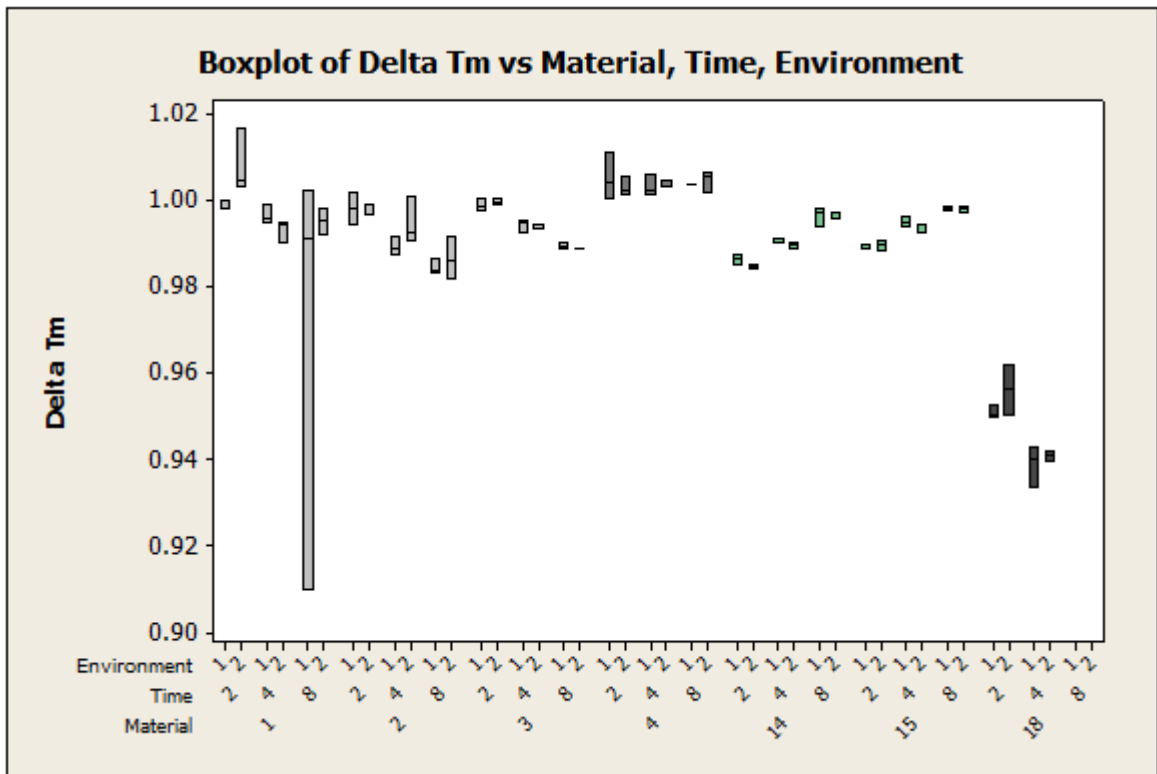


Figure 44: Boxplot of the change in Tm by material, degradation time and degradation environment

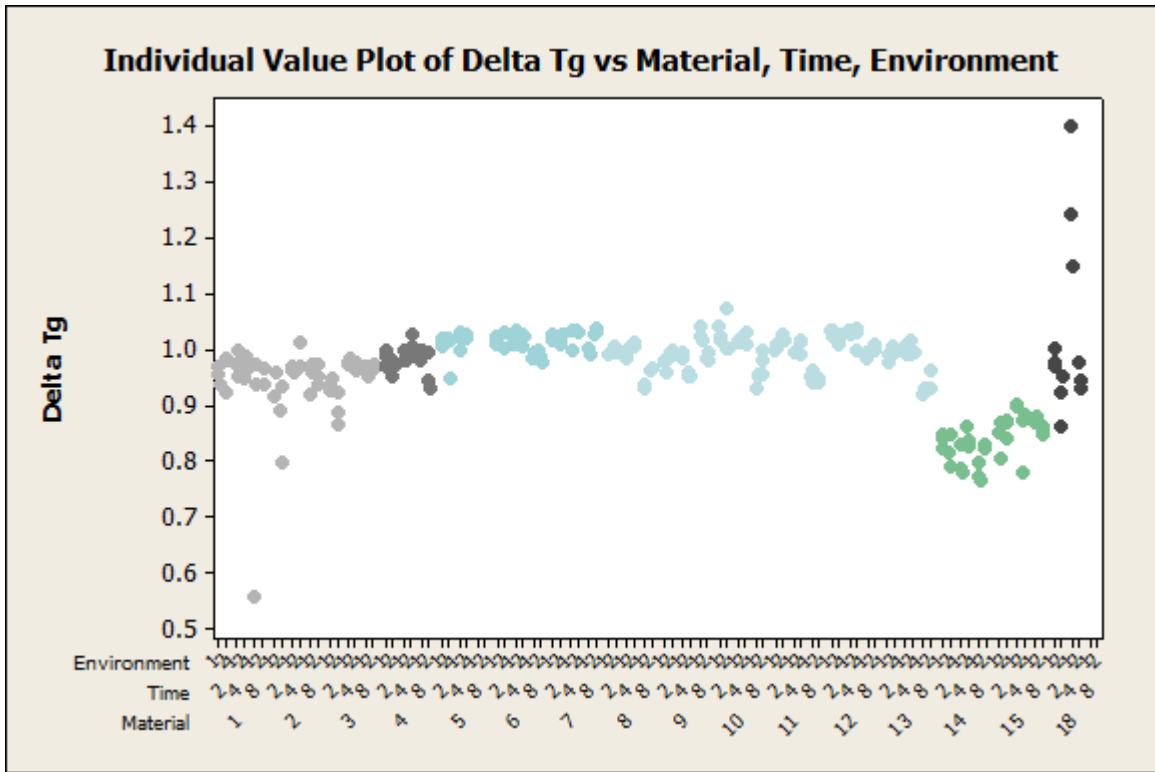


Figure 46: Individual value plot of the change in Tg by material, degradation time and degradation environment

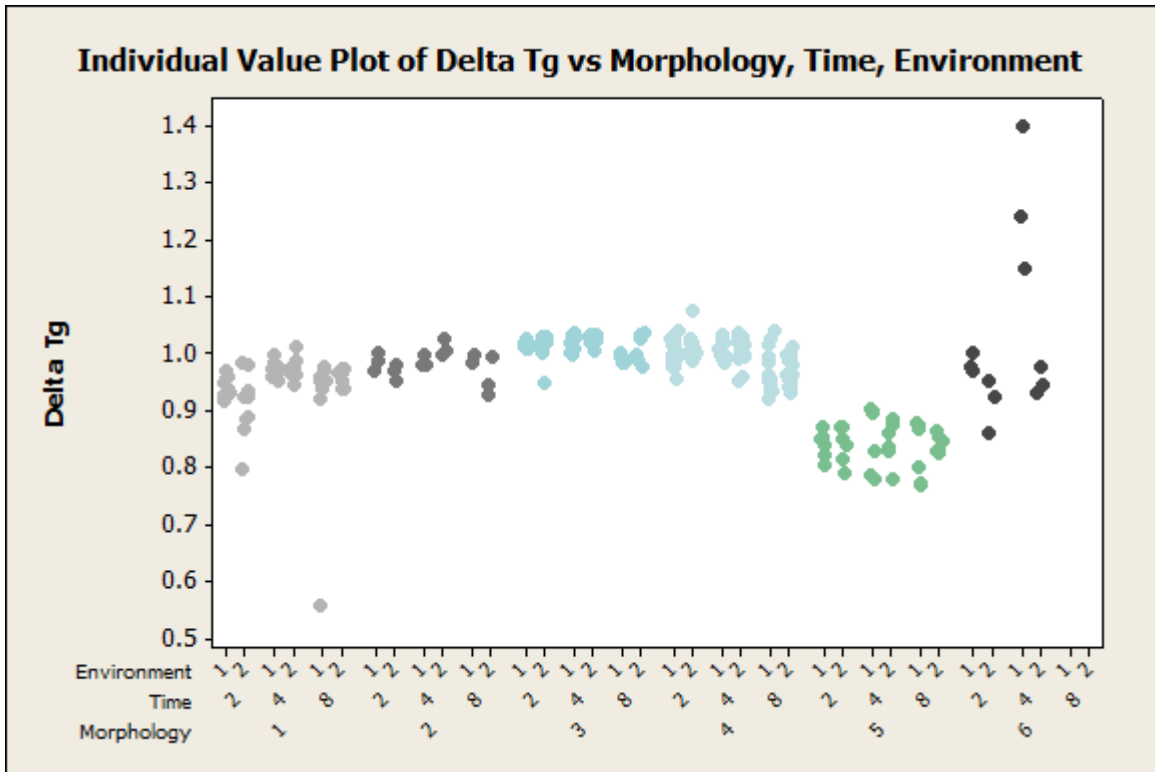


Figure 47: Individual value plot of the change in Tg by morphology, degradation time and degradation environment

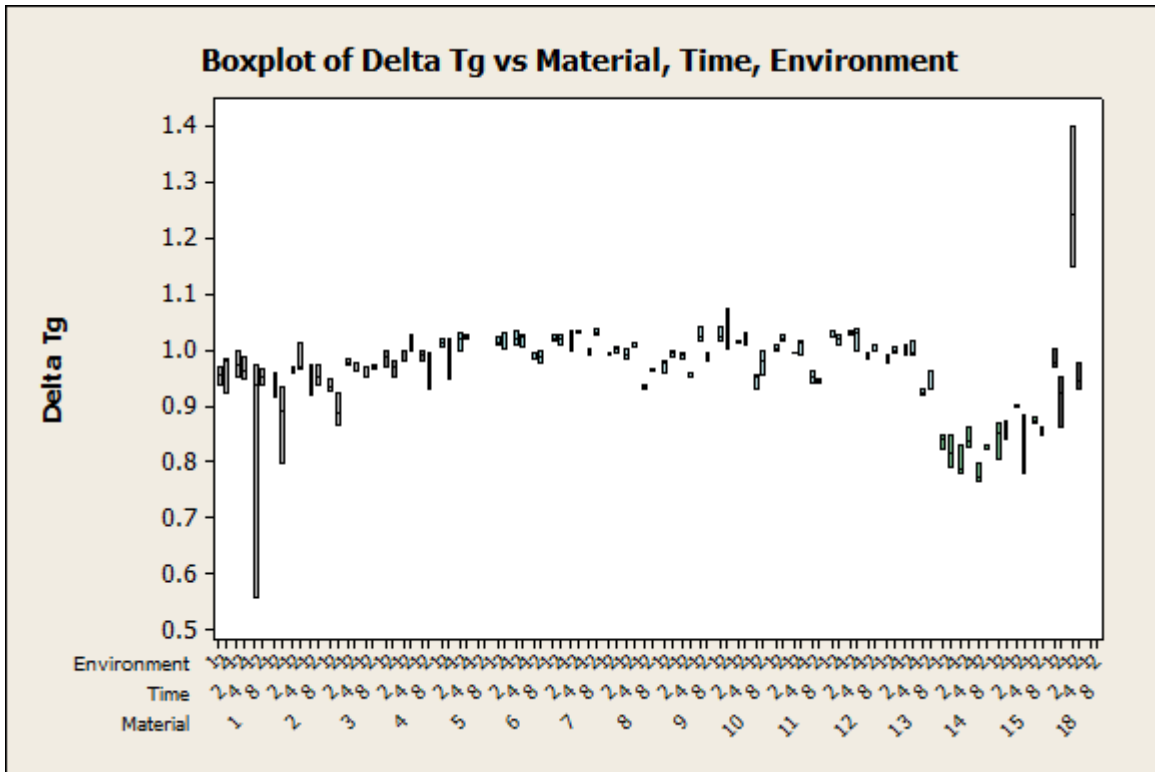


Figure 48: Boxplot of the change in Tg by material, degradation time and degradation environment

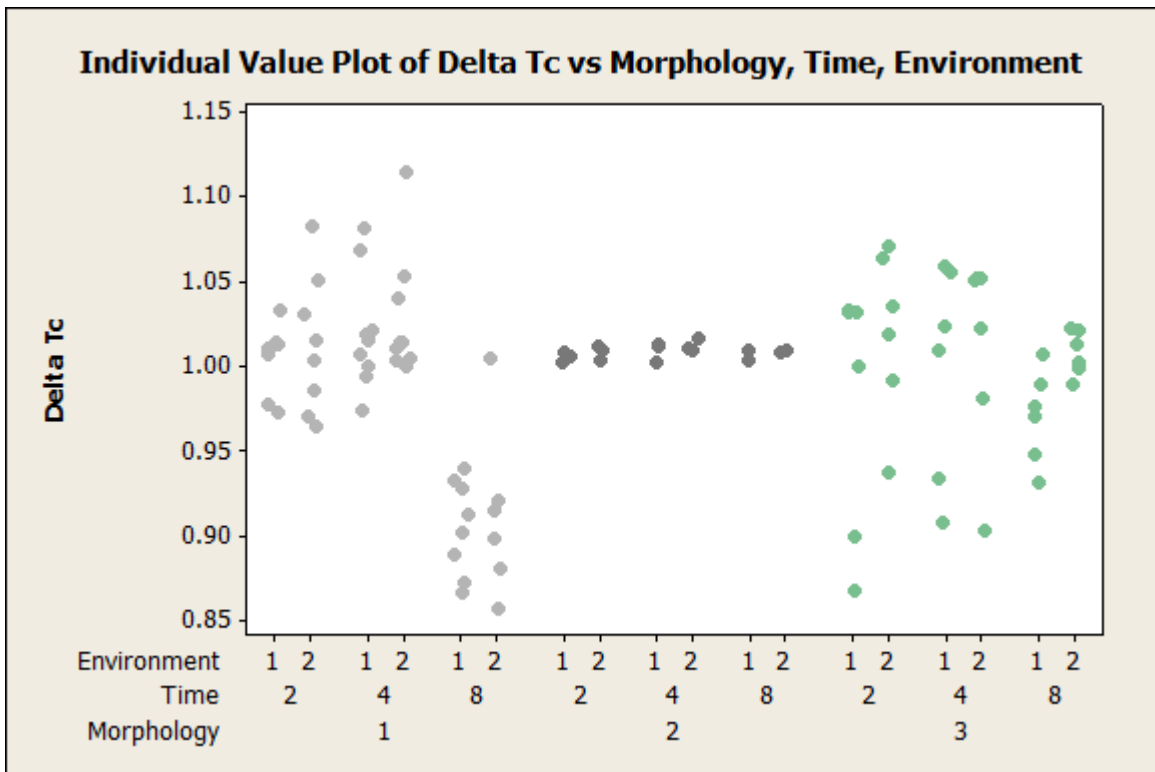


Figure 49: Individual value plot of the change in Tc by morphology, degradation time and degradation environment

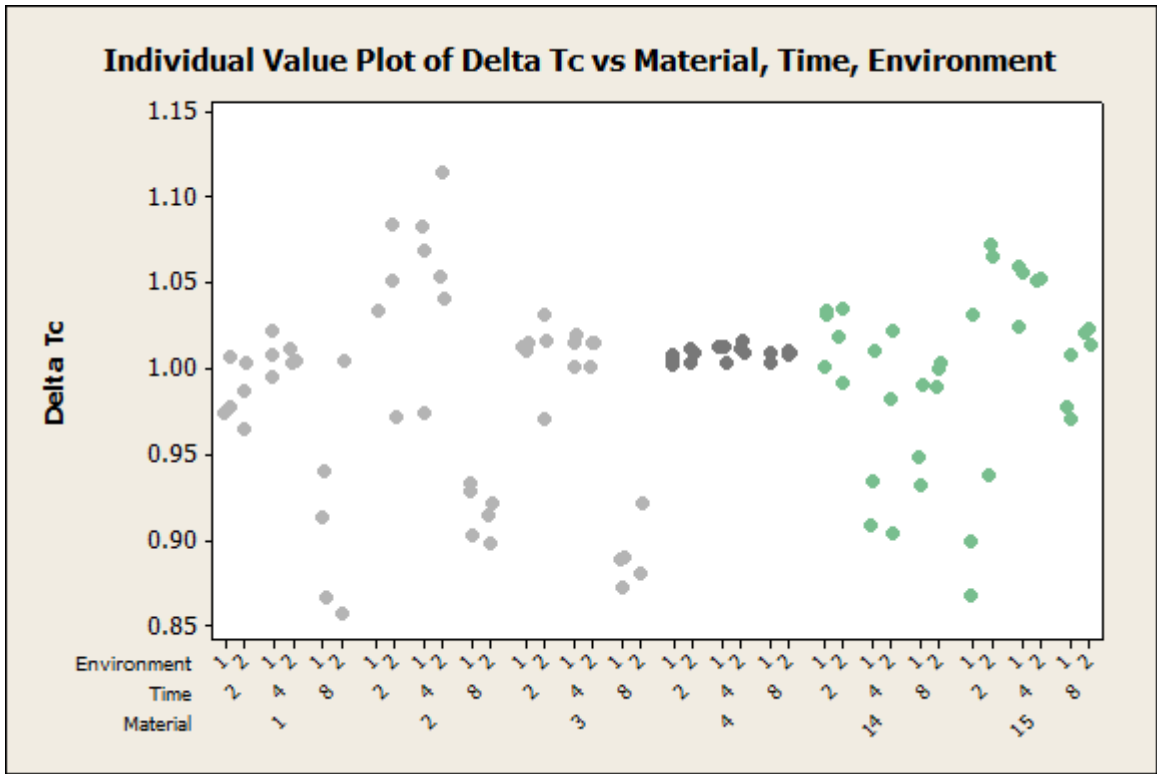


Figure 50: Individual value plot of the change in Tc by material, degradation time and degradation environment

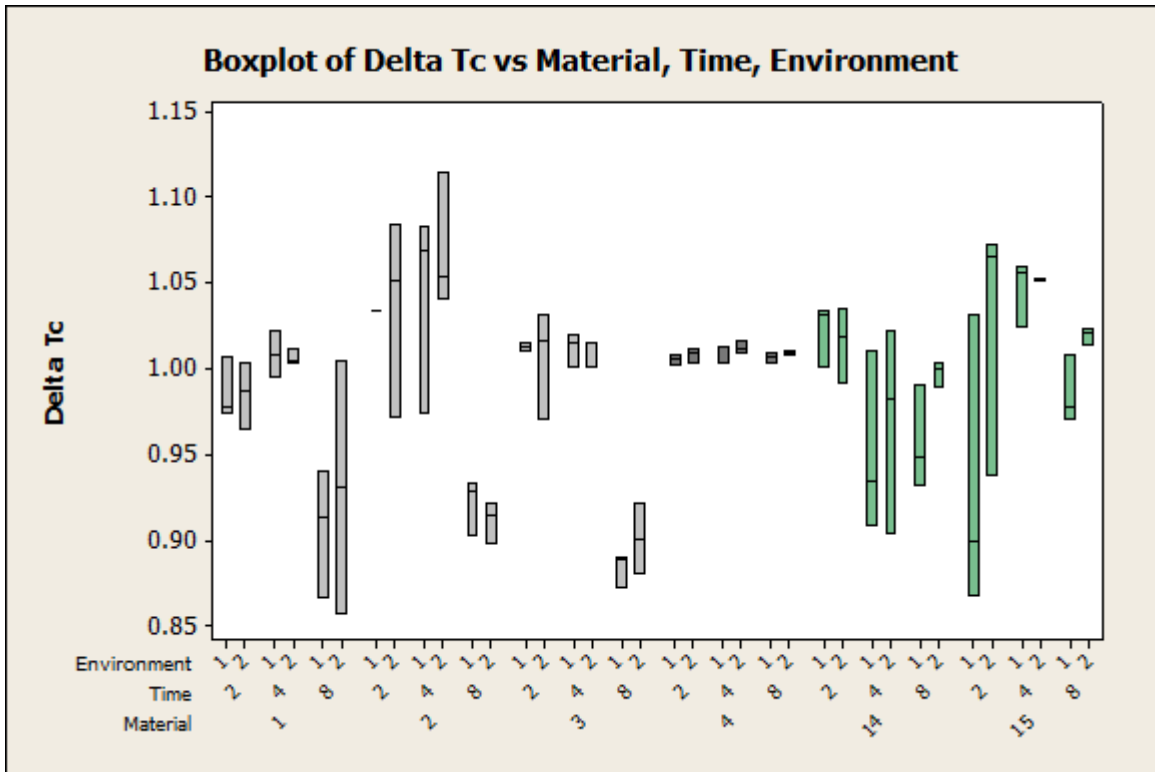


Figure 51: Individual value plot of the change in Tc by material, degradation time and degradation environment

Appendix II: GPC Results

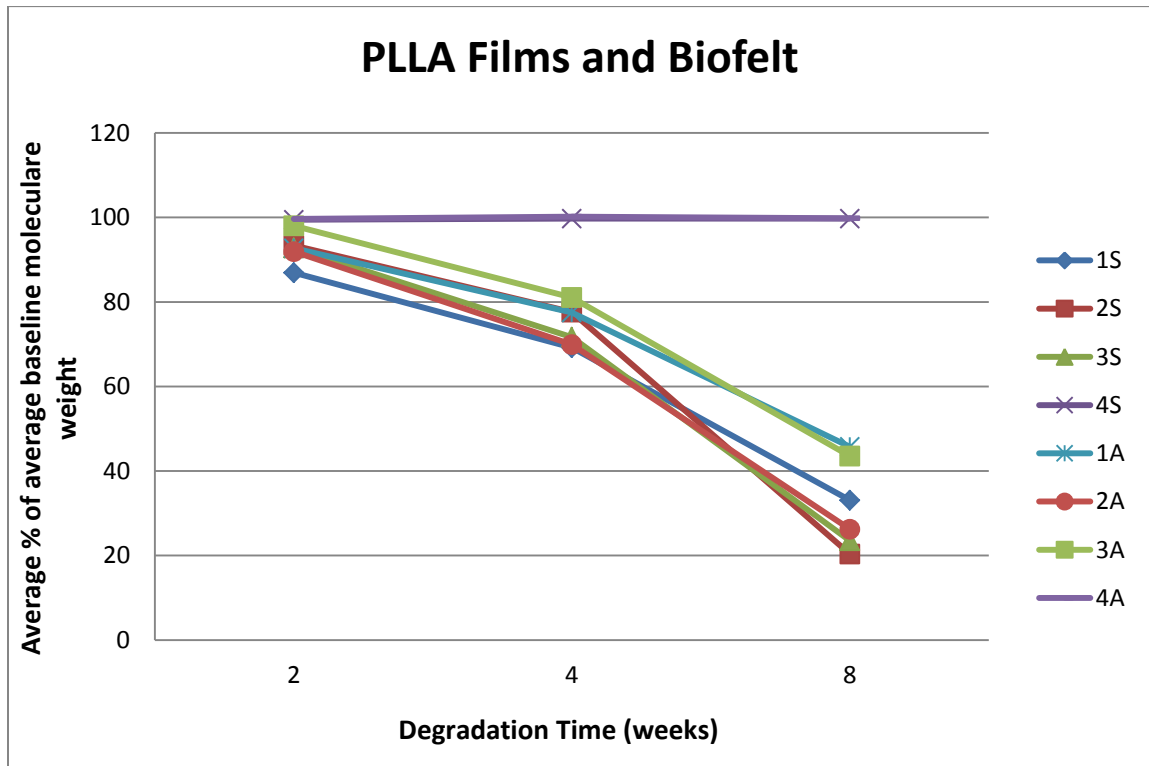


Figure 52: Average percent of average molecular weight at baseline for PLLA films and Biofelt samples

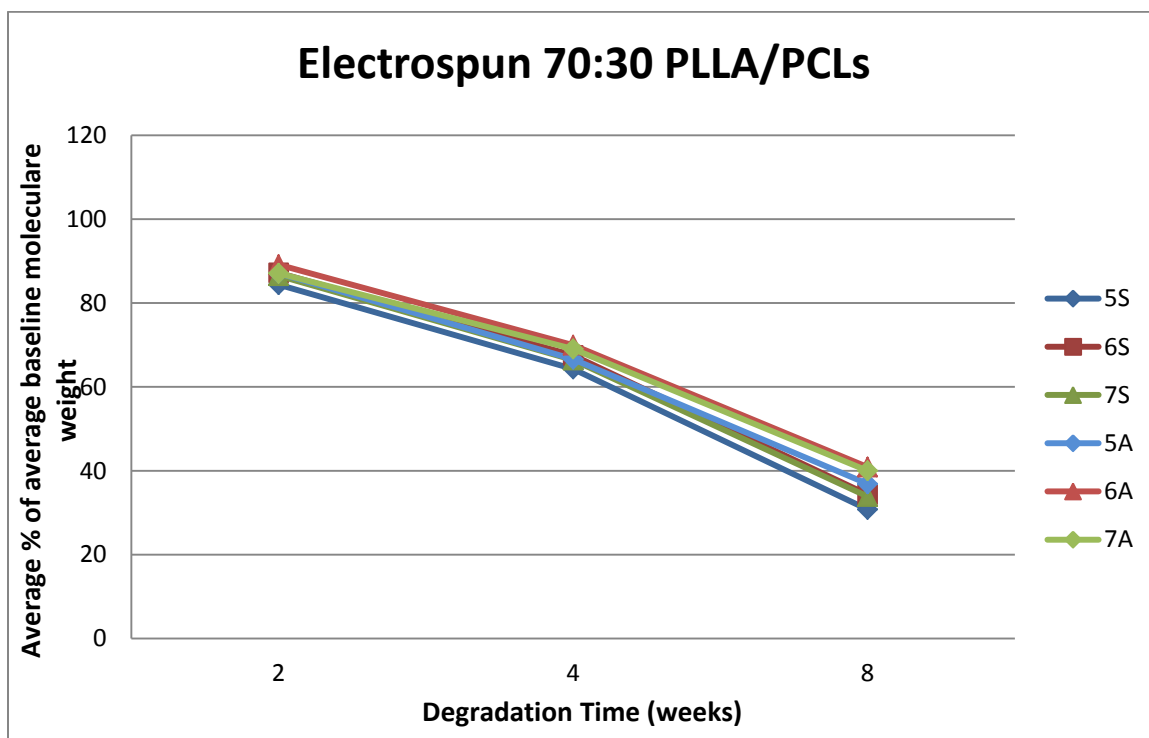


Figure 53: Average percent of average molecular weight at baseline for 70:30 PLLA/PCL electrospun samples

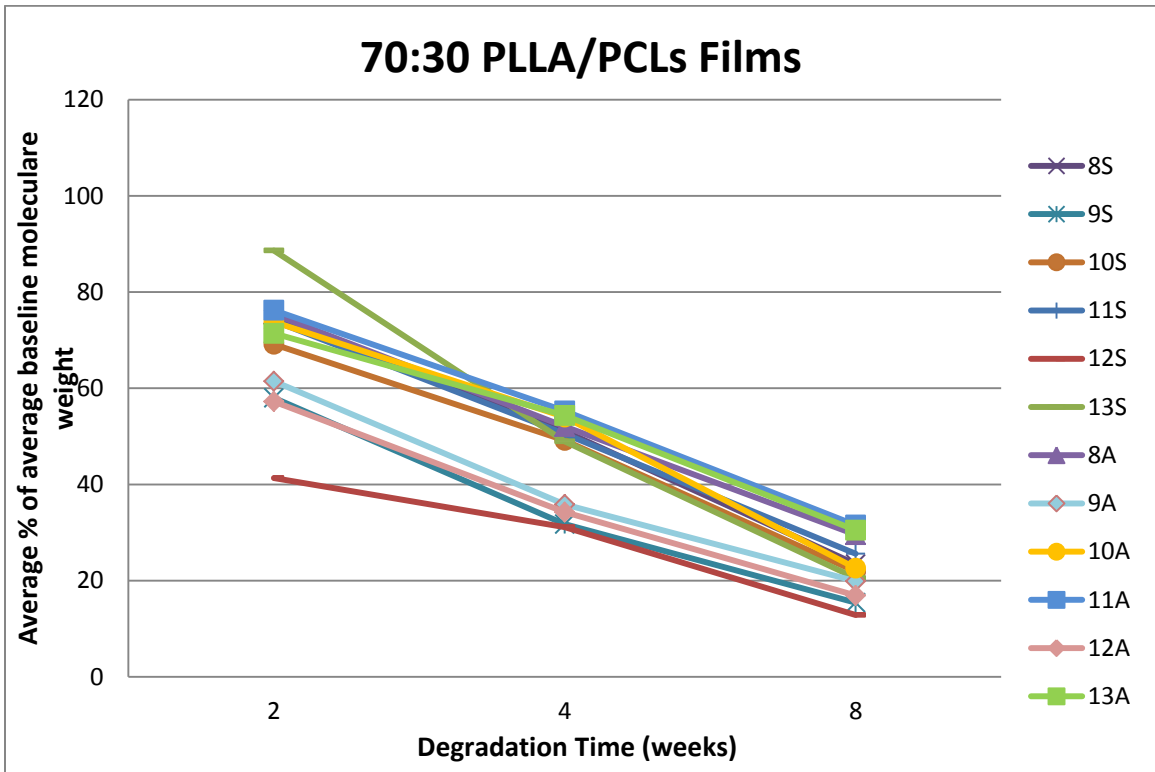


Figure 54: Average percent of average molecular weight at baseline for 70:30 PLLA/PCL film samples.

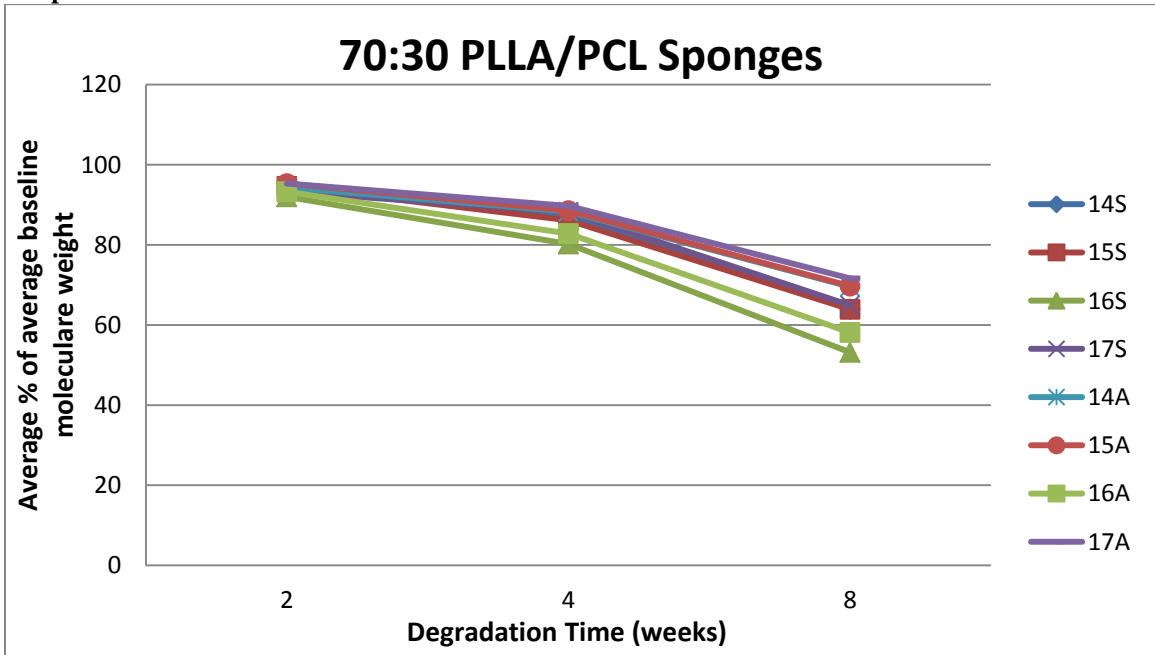


Figure 55: Average percent of average molecular weight at baseline of 70:30 PLLA/PCL sponge samples.

GPC Summary Statistics Output from Minitab:

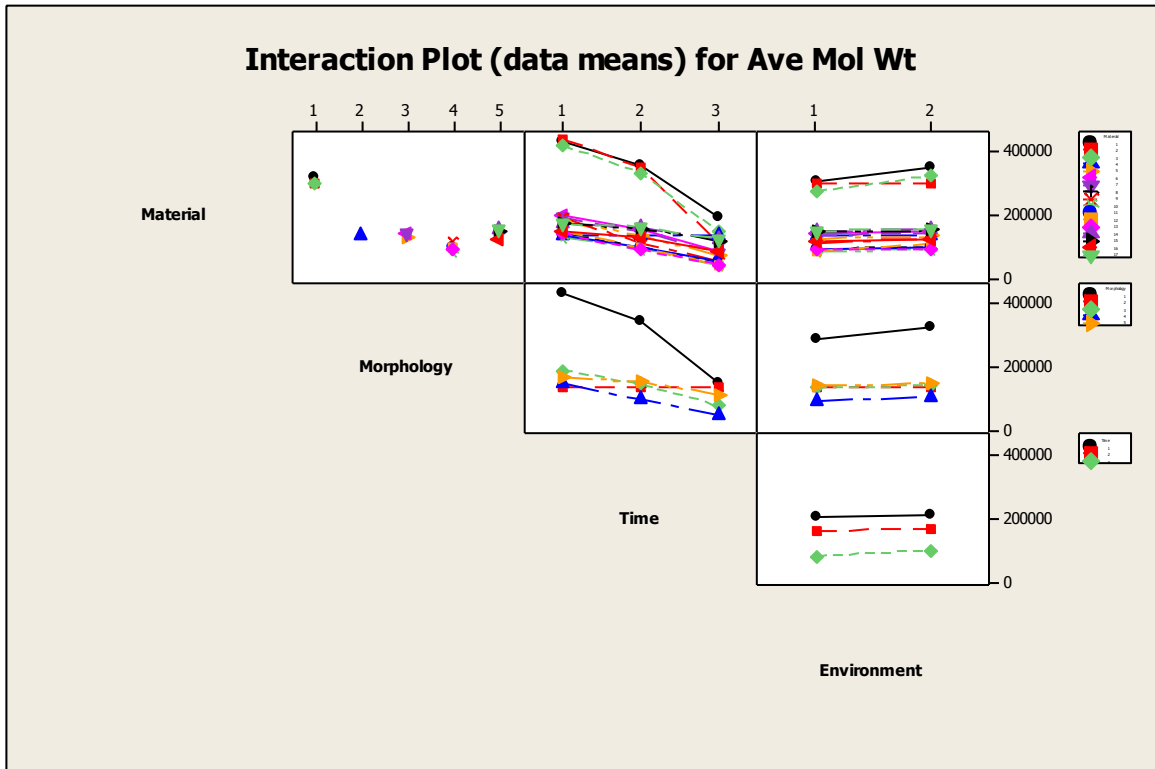


Figure 56: Interaction plot for the proportional molecular weight data, all materials

Descriptive Statistics: Mol Wt Prop

Results for Material = 1, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.8693	0.0273	0.0473	0.8197	0.8197	0.8743
	4	3	0	0.6923	0.0744	0.1288	0.5585	0.5585	0.7029
	8	3	0	0.331	0.144	0.249	0.169	0.169	0.206

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.9140	0.9140
	4	0.8156	0.8156
	8	0.617	0.617

Results for Material = 1, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.9273	0.0185	0.0320	0.8927	0.8927	0.9334
	4	3	0	0.77518	0.00821	0.01421	0.76341	0.76341	0.77116
	8	3	0	0.458	0.104	0.180	0.263	0.263	0.490

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.9558	0.9558
	4	0.79097	0.79097
	8	0.619	0.619

Results for Material = 2, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.9339	0.0119	0.0207	0.9208	0.9208	0.9232
	4	3	0	0.7762	0.0315	0.0546	0.7264	0.7264	0.7675
	8	3	0	0.2036	0.0177	0.0307	0.1687	0.1687	0.2161

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.9577	0.9577
	4	0.8346	0.8346
	8	0.2261	0.2261

Results for Material = 2, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.9193	0.0122	0.0211	0.9017	0.9017	0.9136
	4	3	0	0.69946	0.00842	0.01458	0.68289	0.68289	0.70512
	8	3	0	0.2625	0.0113	0.0195	0.2505	0.2505	0.2520

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.9427	0.9427
	4	0.71037	0.71037
	8	0.2850	0.2850

Results for Material = 3, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.92810	0.00485	0.00840	0.92015	0.92015	0.92727
	4	3	0	0.7169	0.0371	0.0643	0.6599	0.6599	0.7043
	8	3	0	0.2353	0.0266	0.0460	0.1995	0.1995	0.2191

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.93688	0.93688
	4	0.7866	0.7866
	8	0.2872	0.2872

Results for Material = 3, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.9803	0.0316	0.0548	0.9321	0.9321	0.9689
	4	3	0	0.8105	0.0199	0.0344	0.7712	0.7712	0.8249
	8	3	0	0.4353	0.0729	0.1263	0.3273	0.3273	0.4044

Variable	Time	Q3	Maximum
Mol Wt Prop	2	1.0398	1.0398
	4	0.8354	0.8354
	8	0.5741	0.5741

Results for Material = 4, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.99465	0.00291	0.00504	0.98931	0.98931	0.99530
	4	3	0	0.99731	0.00116	0.00201	0.99530	0.99530	0.99731
	8	3	0	0.99732	0.00232	0.00402	0.99330	0.99330	0.99731

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.99932	0.99932
	4	0.99932	0.99932
	8	1.00134	1.00134

Results for Material = 4, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.99665	0.00242	0.00419	0.99330	0.99330	0.99530
	4	3	0	1.0020	0.00441	0.00764	0.9933	0.9933	1.0054
	8	3	0	0.99798	0.00134	0.00232	0.99530	0.99530	0.99932

Variable	Time	Q3	Maximum
Mol Wt Prop	2	1.00134	1.00134
	4	1.0074	1.0074
	8	0.99932	0.99932

Results for Material = 5, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.8440	0.0182	0.0316	0.8088	0.8088	0.8532
	4	3	0	0.64284	0.00489	0.00848	0.63381	0.63381	0.64409
	8	3	0	0.3077	0.0162	0.0280	0.2915	0.2915	0.2915

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.8699	0.8699
	4	0.65063	0.65063
	8	0.3400	0.3400

Results for Material = 5, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.8708	0.0131	0.0228	0.8495	0.8495	0.8680
	4	3	0	0.6661	0.0207	0.0358	0.6250	0.6250	0.6831
	8	3	0	0.3686	0.0175	0.0302	0.3459	0.3459	0.3570

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.8948	0.8948
	4	0.6902	0.6902
	8	0.4030	0.4030

Results for Material = 6, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.87221	0.000638	0.00110	0.87093	0.87093	0.87285
	4	3	0	0.67502	0.00283	0.00490	0.67038	0.67038	0.67454
	8	3	0	0.34333	0.00153	0.00265	0.34151	0.34151	0.34212

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.87285	0.87285
	4	0.68015	0.68015
	8	0.34637	0.34637

Results for Material = 6, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.89166	0.00628	0.01087	0.88443	0.88443	0.88638
	4	3	0	0.6995	0.0114	0.0197	0.6773	0.6773	0.7061
	8	3	0	0.40865	0.00305	0.00528	0.40365	0.40365	0.40812

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.90417	0.90417
	4	0.7150	0.7150
	8	0.41417	0.41417

Results for Material = 7, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.86507	0.00698	0.01209	0.85129	0.85129	0.87005
	4	3	0	0.66396	0.00538	0.00931	0.65668	0.65668	0.66073
	8	3	0	0.33842	0.00200	0.00347	0.33448	0.33448	0.33979

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.87386	0.87386
	4	0.67445	0.67445
	8	0.34099	0.34099

Results for Material = 7, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.87074	0.00647	0.01120	0.86248	0.86248	0.86626
	4	3	0	0.6903	0.0127	0.0221	0.6648	0.6648	0.7015
	8	3	0	0.39984	0.00863	0.01495	0.38421	0.38421	0.40132

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.88349	0.88349
	4	0.7044	0.7044
	8	0.41400	0.41400

Results for Material = 8, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.74313	0.00429	0.00743	0.73459	0.73459	0.74664
	4	3	0	0.51078	0.00290	0.00502	0.50510	0.50510	0.51267

		8	3	0	0.23446	0.00916	0.01587	0.21614	0.21614	0.24323
Variable	Time				Q3	Maximum				
Mol Wt Prop	2				0.74816	0.74816				
	4				0.51459	0.51459				
	8				0.24401	0.24401				

Results for Material = 8, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.75383	0.00593	0.01028	0.74512	0.74512	0.75122
	4	3	0	0.52059	0.00927	0.01606	0.50416	0.50416	0.52136
	8	3	0	0.29567	0.00521	0.00903	0.28631	0.28631	0.29637

Variable	Time		Q3	Maximum
Mol Wt Prop	2		0.76517	0.76517
	4		0.53626	0.53626
	8		0.30433	0.30433

Results for Material = 9, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1
Mol Wt Prop	2	3	0	0.57992	0.00474	0.00822	0.57195	0.57195
	4	3	0	0.31760	0.00145	0.00250	0.31560	0.31560
	8	3	0	0.15408	0.000361	0.000626	0.15342	0.15342

Variable	Time	Median	Q3	Maximum
Mol Wt Prop	2	0.57945	0.58836	0.58836
	4	0.31679	0.32041	0.32041
	8	0.15417	0.15467	0.15467

Results for Material = 9, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.6146	0.0117	0.0203	0.5974	0.5974	0.6094
	4	3	0	0.35839	0.00254	0.00441	0.35330	0.35330	0.36093
	8	3	0	0.19891	0.00396	0.00686	0.19101	0.19101	0.20235

Variable	Time		Q3	Maximum
Mol Wt Prop	2		0.6369	0.6369
	4		0.36093	0.36093
	8		0.20338	0.20338

Results for Material = 10, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.69128	0.00376	0.00651	0.68625	0.68625	0.68897
	4	3	0	0.4914	0.0174	0.0301	0.4571	0.4571	0.5044
	8	3	0	0.21899	0.00793	0.01374	0.20830	0.20830	0.21418

Variable	Time		Q3	Maximum
Mol Wt Prop	2		0.69863	0.69863

4	0.5128	0.5128
8	0.23448	0.23448

Results for Material = 10, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.7380	0.0180	0.0311	0.7028	0.7028	0.7495
	4	3	0	0.5402	0.0105	0.0181	0.5244	0.5244	0.5363
	8	3	0	0.2261	0.0326	0.0565	0.1801	0.1801	0.2089

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.7618	0.7618
	4	0.5600	0.5600
	8	0.2892	0.2892

Results for Material = 11, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.74021	0.00451	0.00781	0.73570	0.73570	0.73570
	4	3	0	0.50529	0.00729	0.01262	0.49289	0.49289	0.50485
	8	3	0	0.25582	0.000625	0.00108	0.25500	0.25500	0.25541

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.74922	0.74922
	4	0.51812	0.51812
	8	0.25705	0.25705

Results for Material = 11, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.76215	0.00896	0.01552	0.74619	0.74619	0.76305
	4	3	0	0.55273	0.00931	0.01613	0.54291	0.54291	0.54393
	8	3	0	0.31559	0.00395	0.00684	0.30896	0.30896	0.31518

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.77720	0.77720
	4	0.57135	0.57135
	8	0.32263	0.32263

Results for Material = 12, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.41317	0.00952	0.01648	0.39414	0.39414	0.42227
	4	3	0	0.31136	0.00566	0.00980	0.30182	0.30182	0.31085
	8	3	0	0.12848	0.00495	0.00857	0.11859	0.11859	0.13312

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.42311	0.42311
	4	0.32140	0.32140
	8	0.13374	0.13374

Results for Material = 12, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.5723	0.0160	0.0278	0.5419	0.5419	0.5787
	4	3	0	0.3424	0.0112	0.0194	0.3268	0.3268	0.3362
	8	3	0	0.16905	0.00834	0.01444	0.15432	0.15432	0.16966

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.5963	0.5963
	4	0.3641	0.3641
	8	0.18318	0.18318

Results for Material = 13, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.88632	0.00326	0.00564	0.88140	0.88140	0.88508
	4	3	0	0.4896	0.0150	0.0260	0.4717	0.4717	0.4777
	8	3	0	0.2055	0.0104	0.0180	0.1928	0.1928	0.1976

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.89248	0.89248
	4	0.5193	0.5193
	8	0.2262	0.2262

Results for Material = 13, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.7141	0.0200	0.0347	0.6743	0.6743	0.7296
	4	3	0	0.5436	0.0127	0.0220	0.5193	0.5193	0.5489
	8	3	0	0.3048	0.0189	0.0327	0.2672	0.2672	0.3209

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.7384	0.7384
	4	0.5624	0.5624
	8	0.3263	0.3263

Results for Material = 14, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.94767	0.00511	0.00886	0.94158	0.94158	0.94359
	4	3	0	0.87437	0.00184	0.00318	0.87254	0.87254	0.87254
	8	3	0	0.63959	0.00253	0.00438	0.63544	0.63544	0.63916

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.95783	0.95783
	4	0.87805	0.87805
	8	0.64417	0.64417

Results for Material = 14, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.94361	0.00404	0.00700	0.93957	0.93957	0.93957
	4	3	0	0.88300	0.00328	0.00568	0.87805	0.87805	0.88174
	8	3	0	0.69568	0.00627	0.01086	0.68874	0.68874	0.69011

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.95169	0.95169
	4	0.88920	0.88920
	8	0.70820	0.70820

Results for Material = 15, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.94643	0.00308	0.00534	0.94240	0.94240	0.94441
	4	3	0	0.86146	0.000598	0.00104	0.86086	0.86086	0.86086
	8	3	0	0.63933	0.00378	0.00655	0.63191	0.63191	0.64179

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.95249	0.95249
	4	0.86266	0.86266
	8	0.64429	0.64429

Results for Material = 15, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.95318	0.00296	0.00512	0.94844	0.94844	0.95249
	4	3	0	0.88581	0.00223	0.00387	0.88271	0.88271	0.88456
	8	3	0	0.6972	0.0117	0.0202	0.6739	0.6739	0.7067

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.95860	0.95860
	4	0.89015	0.89015
	8	0.7109	0.7109

Results for Material = 16, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.9201	0.0105	0.0182	0.9037	0.9037	0.9168
	4	3	0	0.8020	0.0179	0.0310	0.7700	0.7700	0.8042
	8	3	0	0.53193	0.00406	0.00704	0.52397	0.52397	0.53445

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.9396	0.9396
	4	0.8319	0.8319
	8	0.53736	0.53736

Results for Material = 16, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.93269	0.00887	0.01536	0.91677	0.91677	0.93386
	4	3	0	0.82804	0.00387	0.00670	0.82197	0.82197	0.82691
	8	3	0	0.58154	0.00199	0.00345	0.57904	0.57904	0.58010

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.94742	0.94742
	4	0.83523	0.83523
	8	0.58547	0.58547

Results for Material = 17, Environment = 1

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.93229	0.00414	0.00717	0.92634	0.92634	0.93029
	4	3	0	0.88239	0.00284	0.00492	0.87868	0.87868	0.88053
	8	3	0	0.64927	0.00224	0.00388	0.64589	0.64589	0.64842

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.94025	0.94025
	4	0.88797	0.88797
	8	0.65351	0.65351

Results for Material = 17, Environment = 2

Variable	Time	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Mol Wt Prop	2	3	0	0.95307	0.00245	0.00424	0.94831	0.94831	0.95442
	4	3	0	0.89740	0.00475	0.00823	0.88797	0.88797	0.90117
	8	3	0	0.71634	0.00456	0.00790	0.70871	0.70871	0.71583

Variable	Time	Q3	Maximum
Mol Wt Prop	2	0.95647	0.95647
	4	0.90308	0.90308
	8	0.72449	0.72449

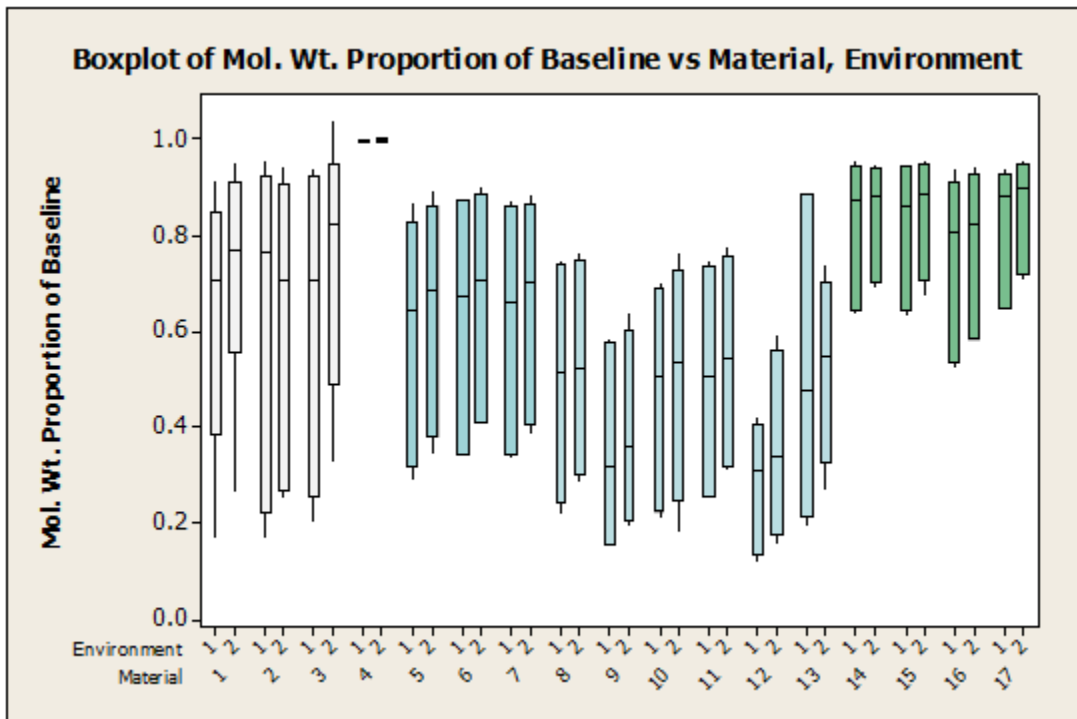


Figure 57: Boxplot of the proportion of molecular weight at baseline by material and degradation environment

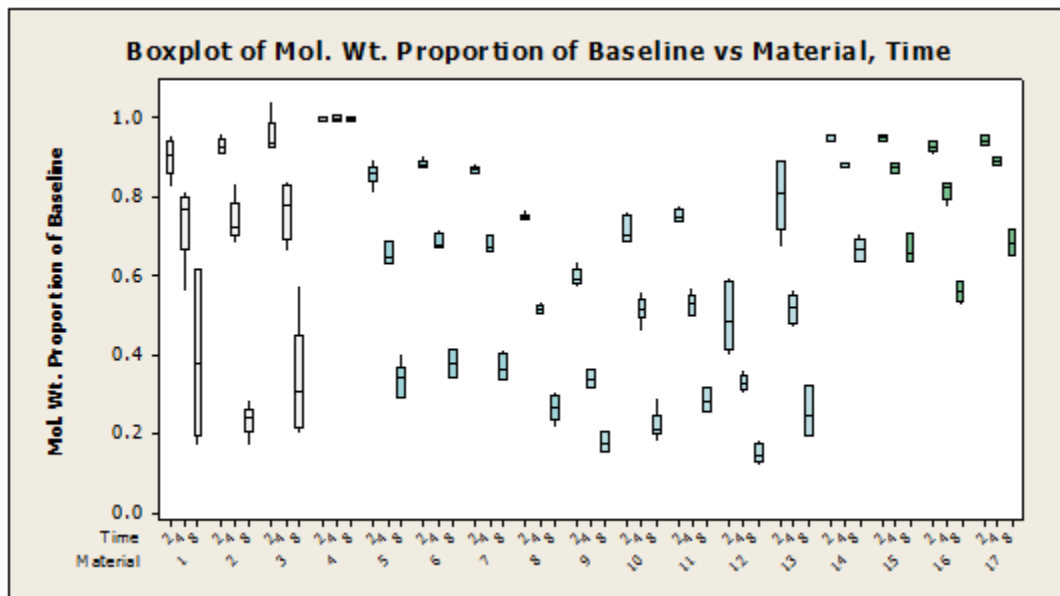


Figure 58: Boxplot of the proportion of molecular weight at baseline by material and degradation time

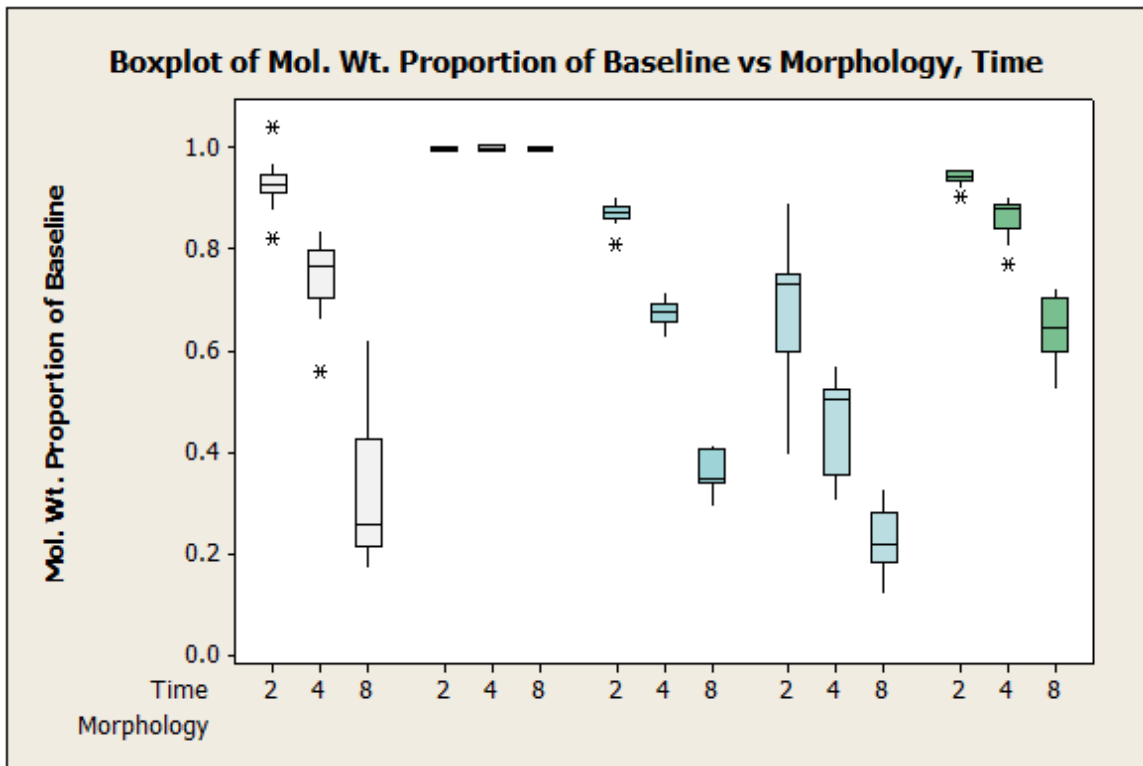


Figure 59: Boxplot of the proportion of molecular weight at baseline by morphology and degradation time

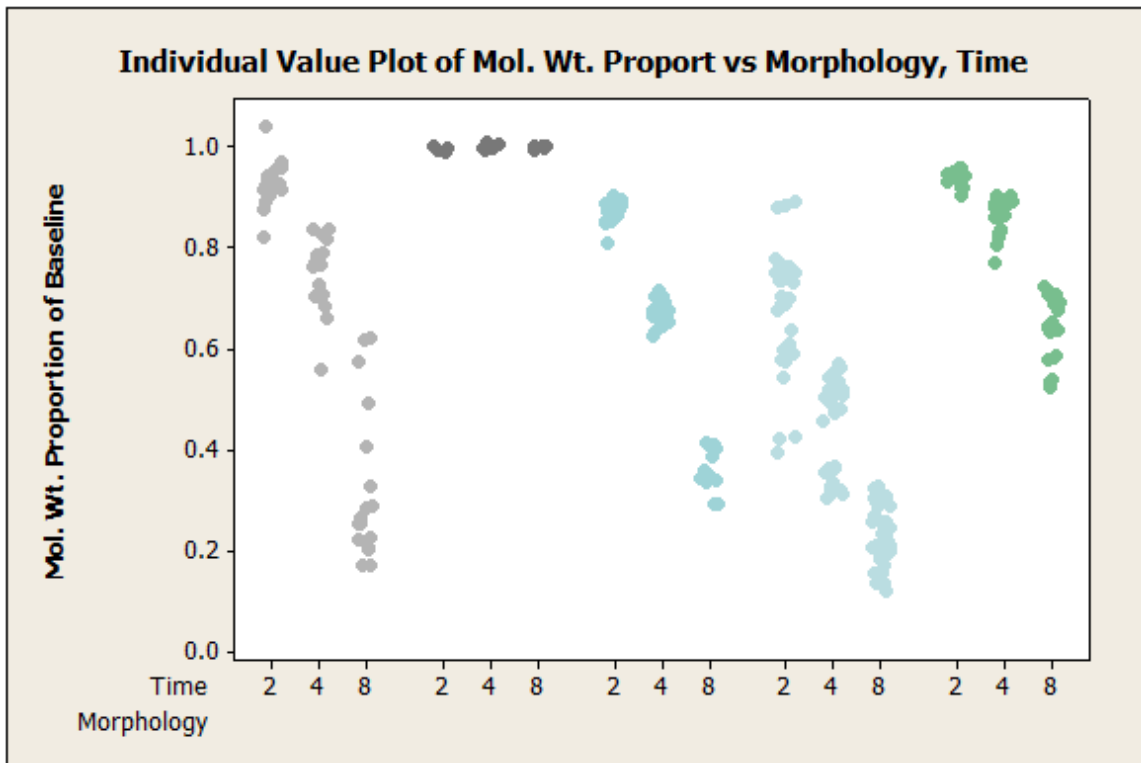


Figure 60:P Individual value plot of the proportion of the baseline molecular weight by morphology and degradation time

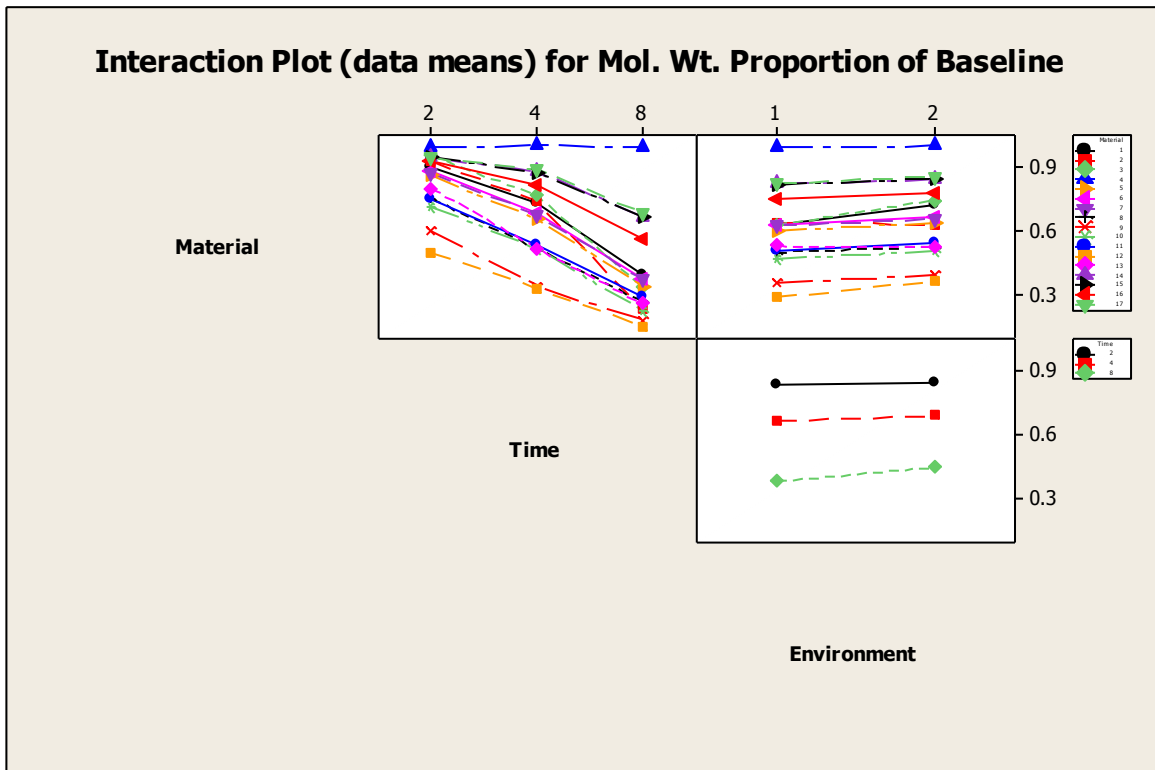


Figure 61: Interaction plot for the proportional molecular weight data, all materials

ANOVA: Mol. Wt. Proport versus Material, Time, Environment

Factor	Type	Levels
Material	fixed	17
Time	fixed	3
Environment	fixed	2

Factor	Values
Material	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	2, 4, 8
Environment	1, 2

Analysis of Variance for Mol. Wt. Proportion of Baseline

Source	DF	SS	MS	F	P
Material	16	9.02785	0.56424	304.57	0.000
Time	2	9.51510	4.75755	2568.07	0.000
Environment	1	0.10486	0.10486	56.60	0.000
Material*Time	32	1.42988	0.04468	24.12	0.000
Material*Environment	16	0.07369	0.00461	2.49	0.002
Time*Environment	2	0.03324	0.01662	8.97	0.000
Error	236	0.43721	0.00185		
Total	305	20.62184			

S = 0.0430416 R-Sq = 97.88% R-Sq(adj) = 97.26%

Source	Variance component	Error term	Expected Mean Square for Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 102 Q[2]
3 Environment		7	(7) + 153 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 51 Q[6]
7 Error	0.00185		(7)

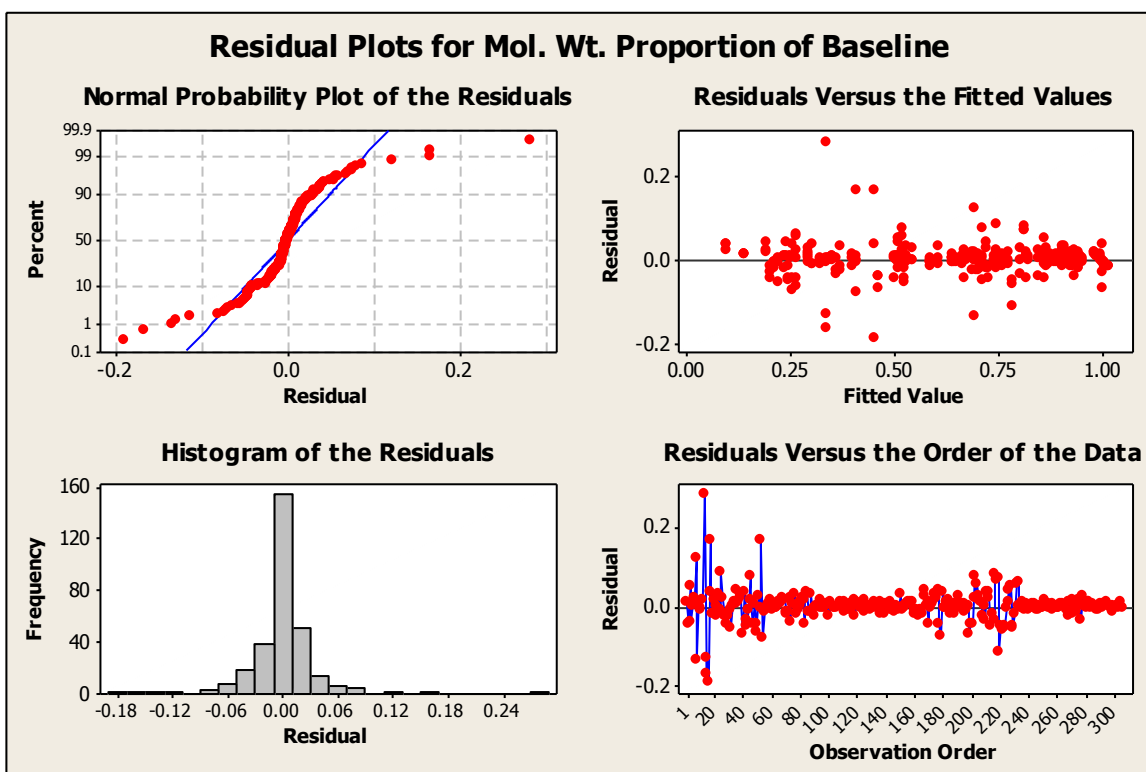


Figure 62: Residual plots for proportional molecular weight data, all materials

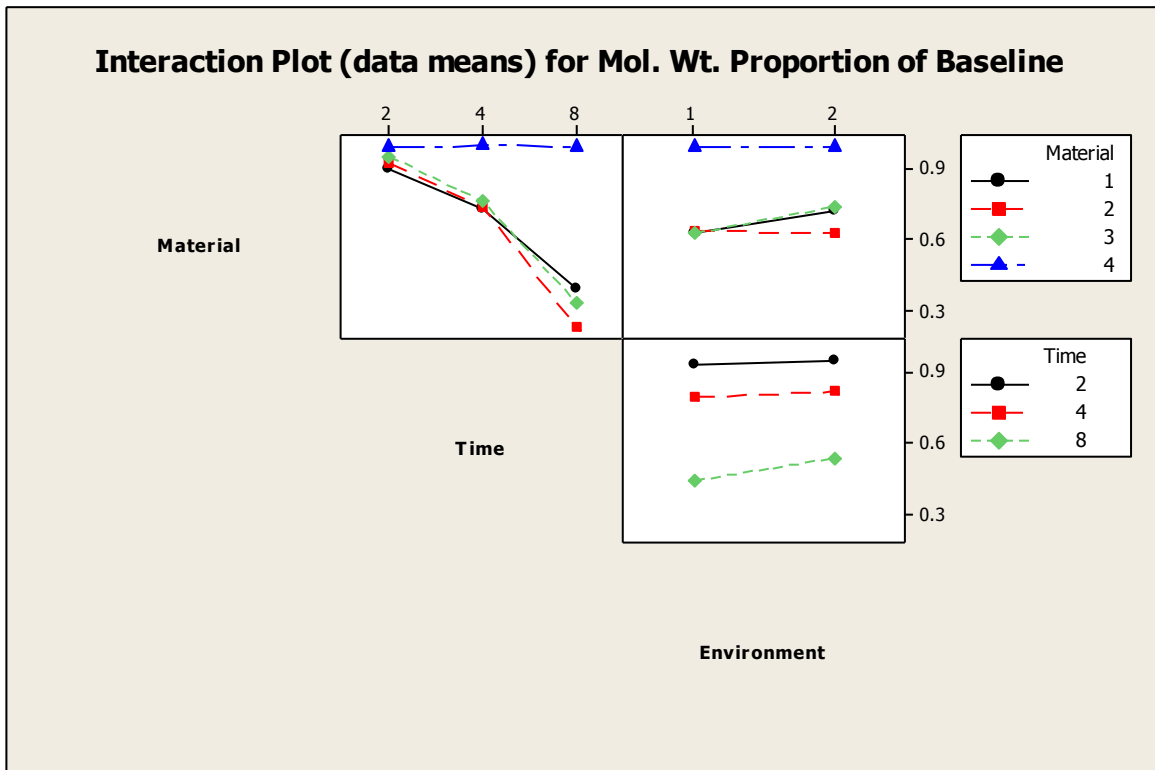


Figure 63: Interaction plot for the proportional molecular weight data, PLLA materials 1-4

ANOVA: Mol. Wt. Proport versus Material, Time, Environment

Factor	Type	Levels	Values
Material	fixed	4	1, 2, 3, 4
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Mol. Wt. Proportion of Baseline

Source	DF	SS	MS	F	P
Material	3	1.52978	0.50993	89.60	0.000
Time	2	2.60473	1.30237	228.84	0.000
Environment	1	0.04326	0.04326	7.60	0.008
Material*Time	6	0.93721	0.15620	27.45	0.000
Material*Environment	3	0.05285	0.01762	3.10	0.034
Time*Environment	2	0.02035	0.01018	1.79	0.177
Error	54	0.30732	0.00569		
Total	71	5.49550			

S = 0.0754400 R-Sq = 94.41% R-Sq(adj) = 92.65%

Expected Mean
Square for
Each Term

Source	Variance component	Error term	(using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 24 Q[2]
3 Environment		7	(7) + 36 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 12 Q[6]
7 Error	0.00569		(7)

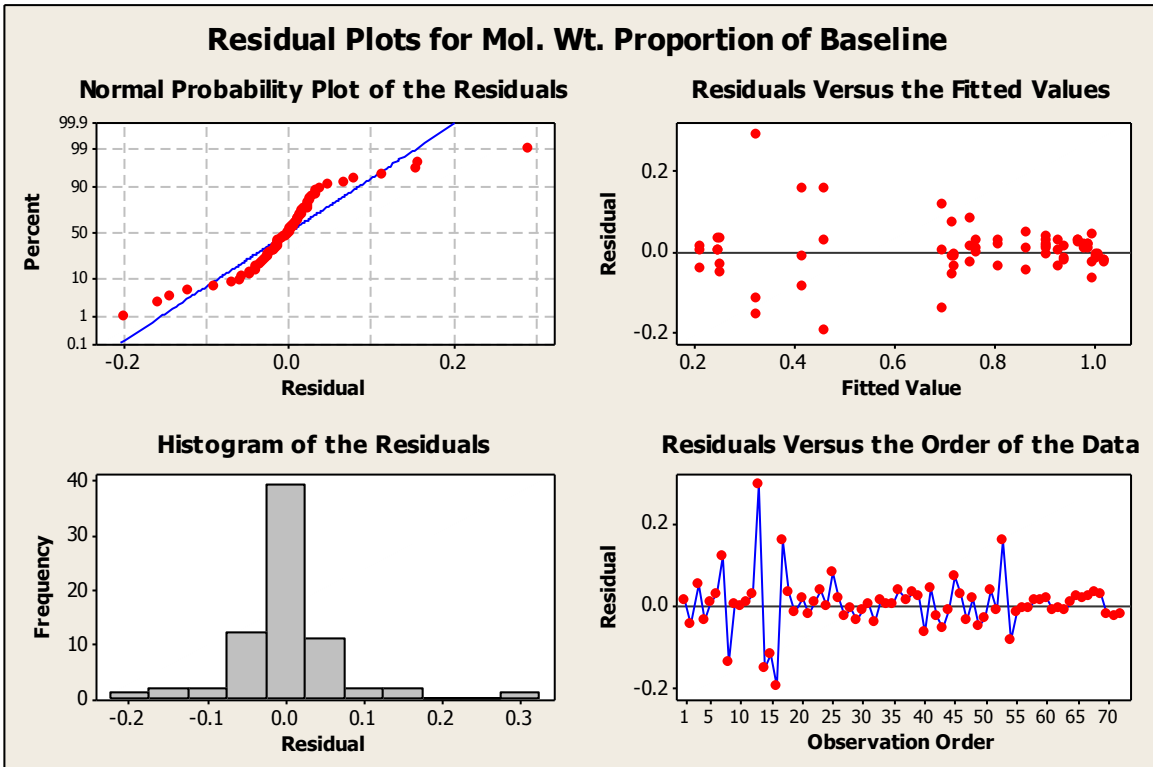


Figure 64: Residual plots for the proportional molecular weight data, PLLA material 1-4

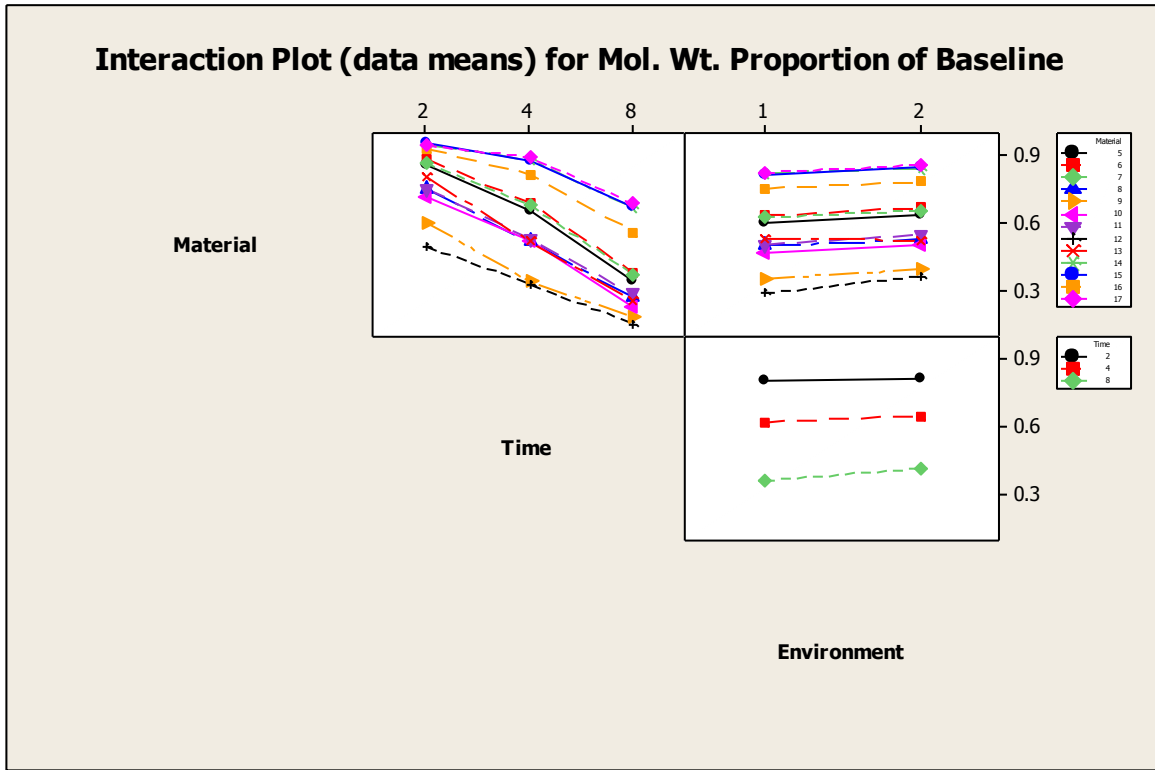


Figure 65: Interaction plots for the proportional molecular weight data, PLLA/PCL material 5-17

ANOVA: Mol. Wt. Proport versus Material, Time, Environment

Factor	Type	Levels	Values
Material	fixed	13	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Mol. Wt. Proportion of Baseline

Source	DF	SS	MS	F	P
Material	12	6.42474	0.53539	769.00	0.000
Time	2	6.95819	3.47910	4997.09	0.000
Environment	1	0.06499	0.06499	93.35	0.000
Material*Time	24	0.44485	0.01854	26.62	0.000
Material*Environment	12	0.01746	0.00145	2.09	0.020
Time*Environment	2	0.01745	0.00872	12.53	0.000
Error	180	0.12532	0.00070		
Total	233	14.05300			

S = 0.0263861 R-Sq = 99.11% R-Sq(adj) = 98.85%

Expected Mean
Square for
Each Term

Source	Variance component	Error term	(using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 78 Q[2]
3 Environment		7	(7) + 117 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 39 Q[6]
7 Error	0.00070		(7)

Residual Plots for Mol. Wt. Proportion of Baseline

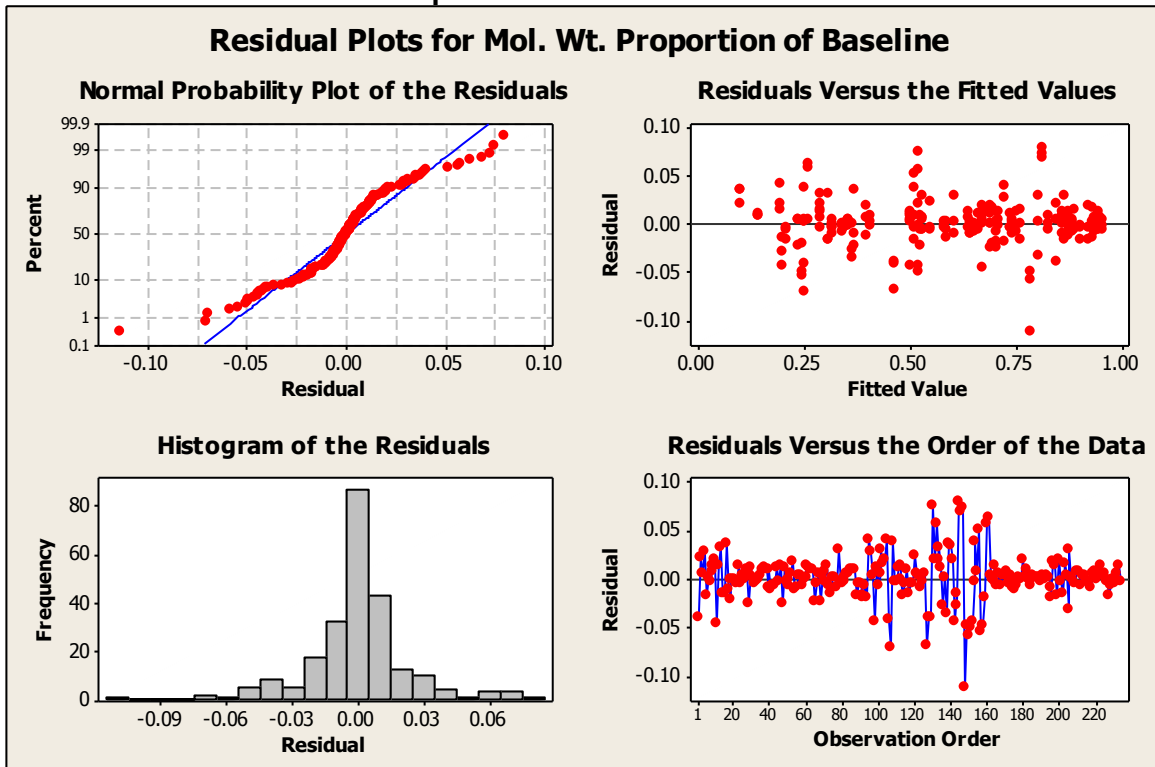


Figure 66: Residual plots for the proportional molecular weight data, PLLA/PCL material 5-17

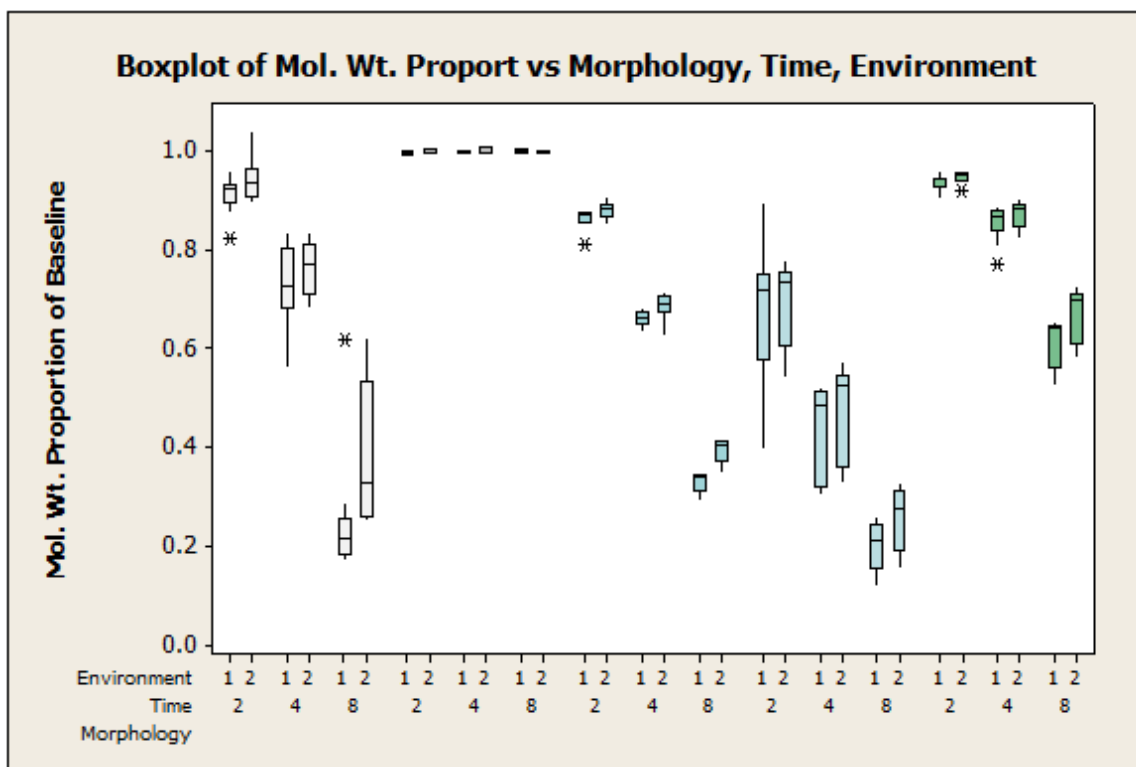


Figure 67: Boxplot of proportional molecular weight by morphology, degradation time and degradation environment

Appendix III: Peak Strain

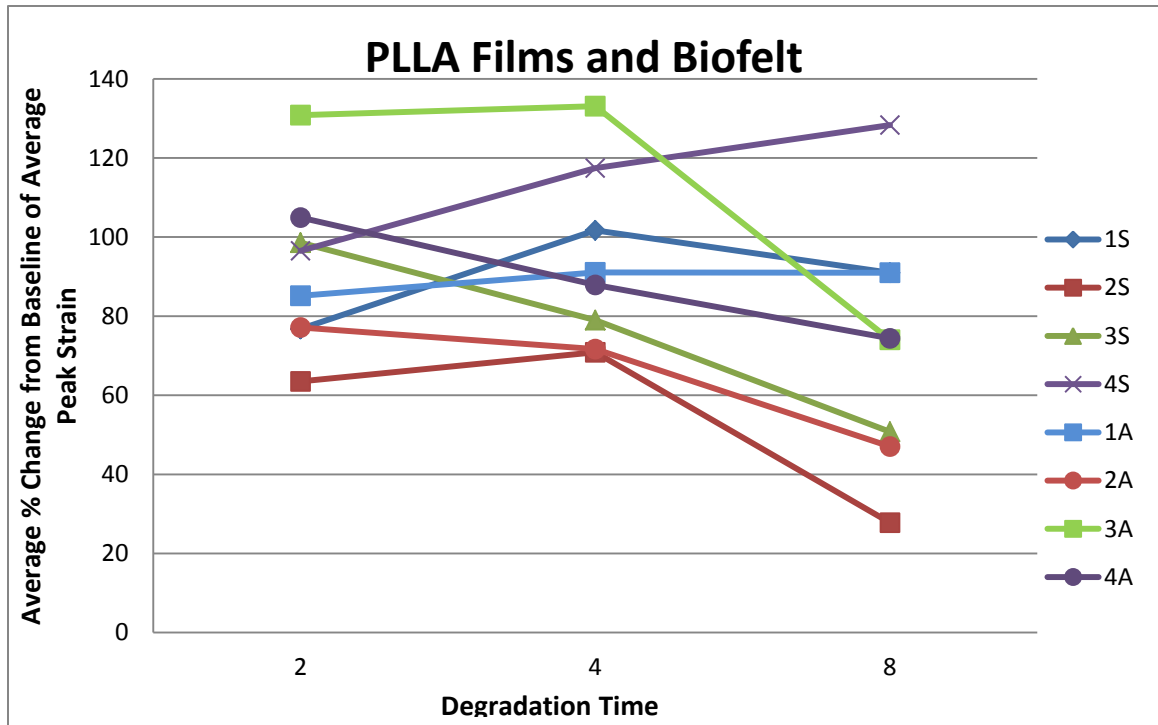


Figure 68: Average percent change from baseline of average peak strain in PLLA films and biofelts (materials 1 through 4). Legend: 1S = material 1 in stagnant environment, 1A = material 1 in agitated environment

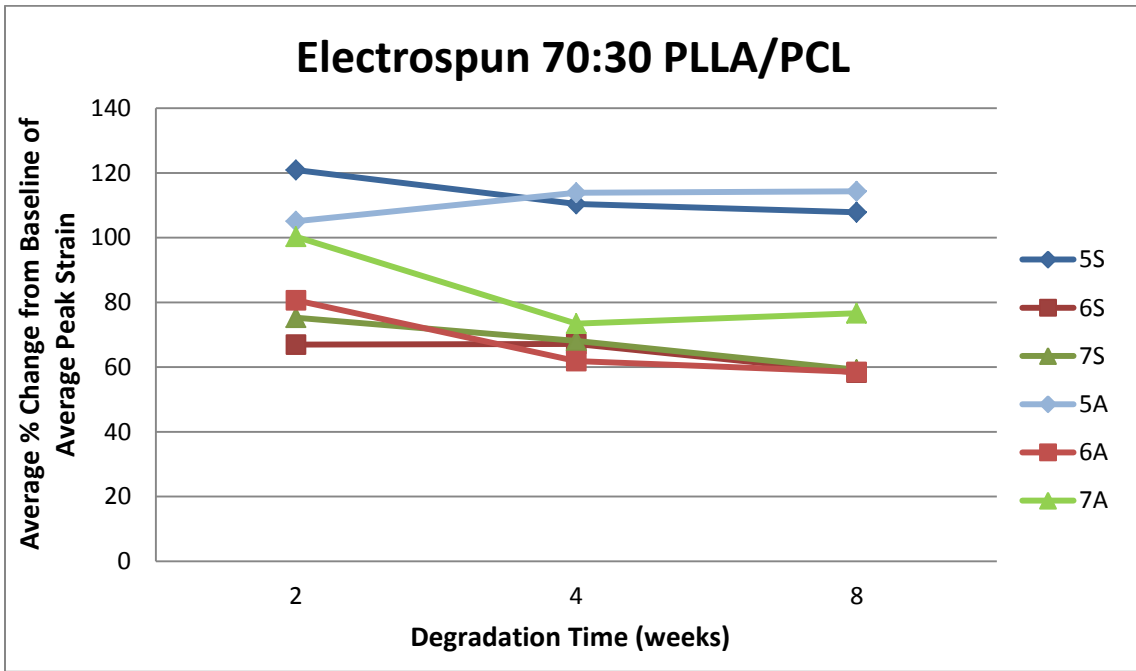


Figure 69: Average percent change from baseline of average peak strain for Electrospun 70:30 PLLA/PCL

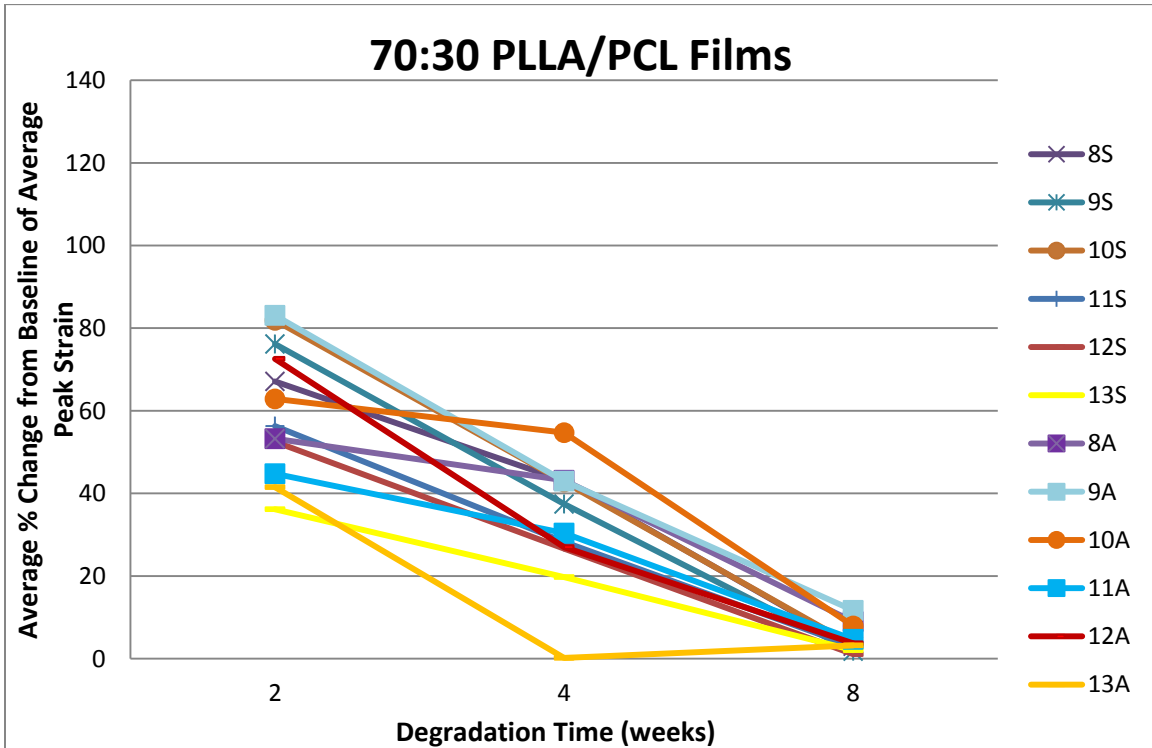


Figure 70: Average percent change from baseline of average peak strain for 70:30 PLLA/PCL films

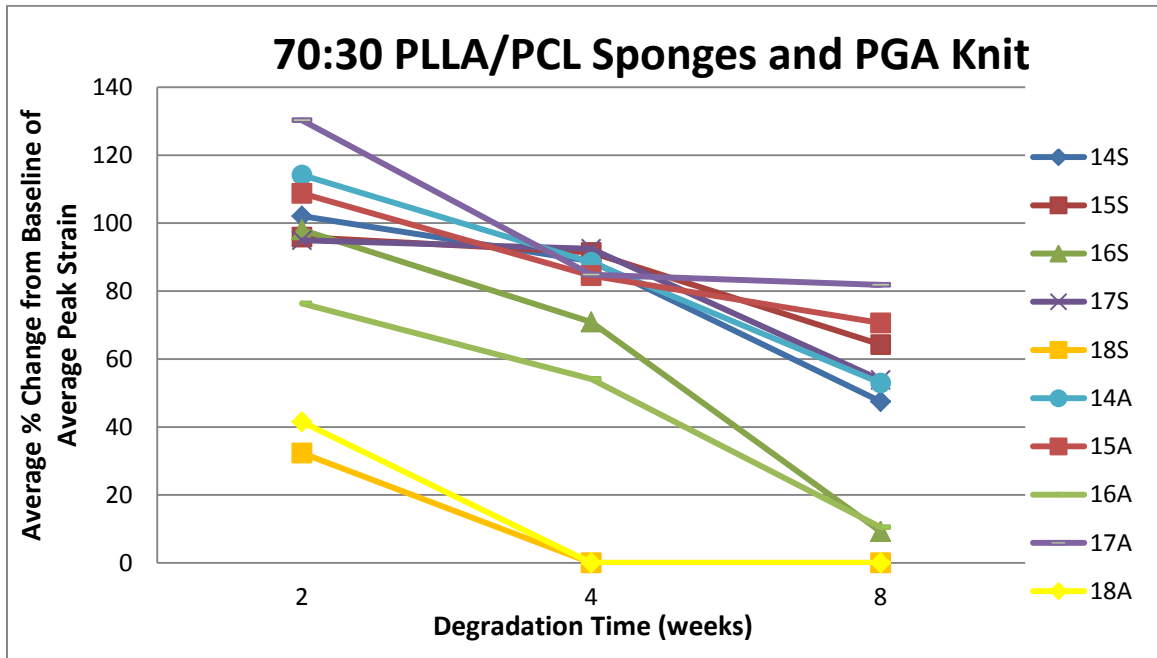


Figure 71: Average percent change from baseline of average peak strain of 70:30 PLLA/PCL sponges (materials 14 through 17) and PGA knit (material 18).

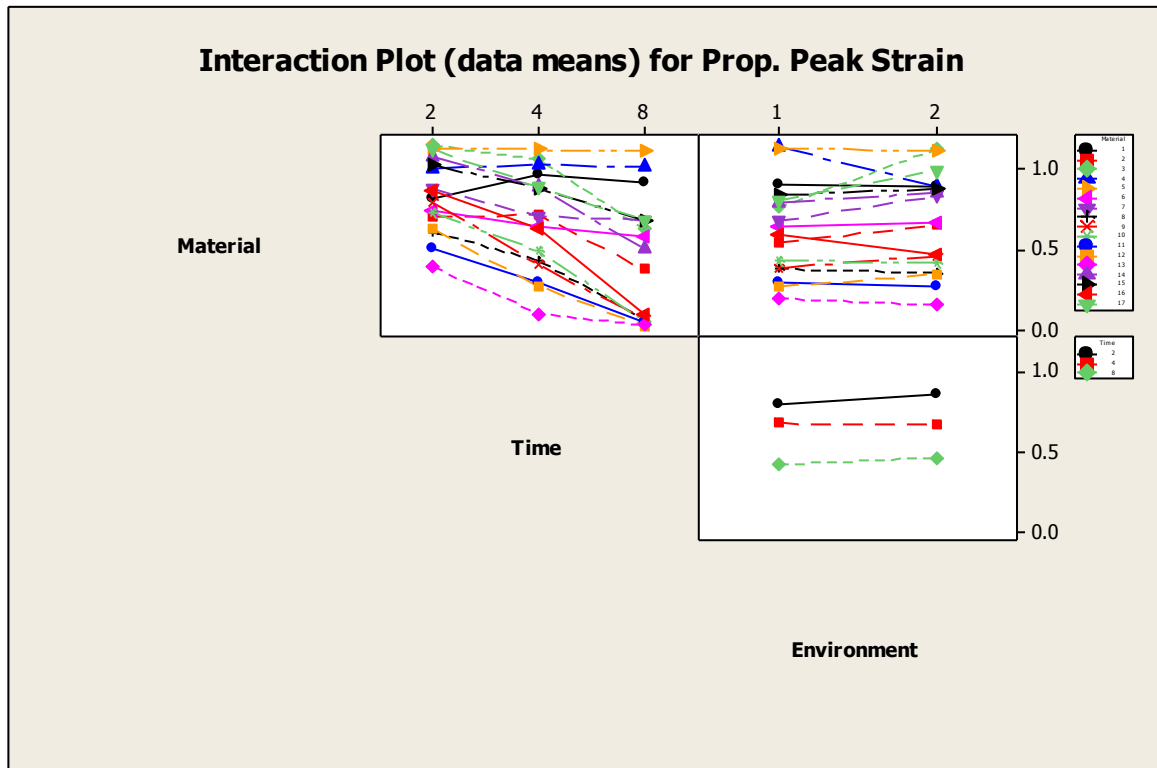


Figure 72: Interaction plots for the proportional peak strain, all materials

ANOVA: Proportion of Peak Strain from Baseline versus Material, Degradation Time and degradation Environment (materials 1-17)

Factor	Type	Levels
Material	fixed	17
Time	fixed	3
Environment	fixed	2

Factor	Values
Material	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	2, 4, 8
Environment	1, 2

Analysis of Variance for Prop. Peak Strain

Source	DF	SS	MS	F	P
Material	16	24.02588	1.50162	50.40	0.000
Time	2	7.87364	3.93682	132.12	0.000
Environment	1	0.09438	0.09438	3.17	0.076
Material*Time	32	3.77511	0.11797	3.96	0.000
Material*Environment	16	1.27755	0.07985	2.68	0.001
Time*Environment	2	0.07417	0.03709	1.24	0.290
Error	236	7.03192	0.02980		
Total	305	44.15266			

S = 0.172616 R-Sq = 84.07% R-Sq(adj) = 79.42%

Source	Variance component	Error term	Expected Mean Square for Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 102 Q[2]
3 Environment		7	(7) + 153 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 51 Q[6]
7 Error	0.02980		(7)

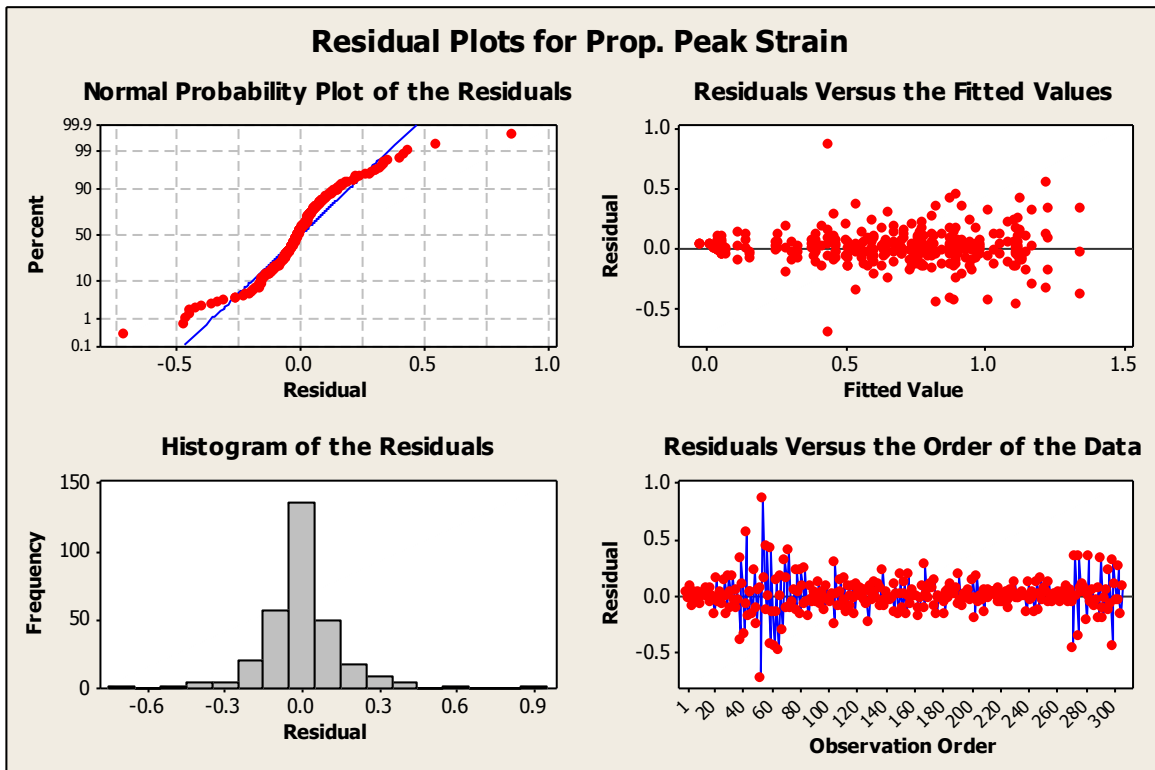


Figure 73: Residual plots for the proportional peak strain, all materials

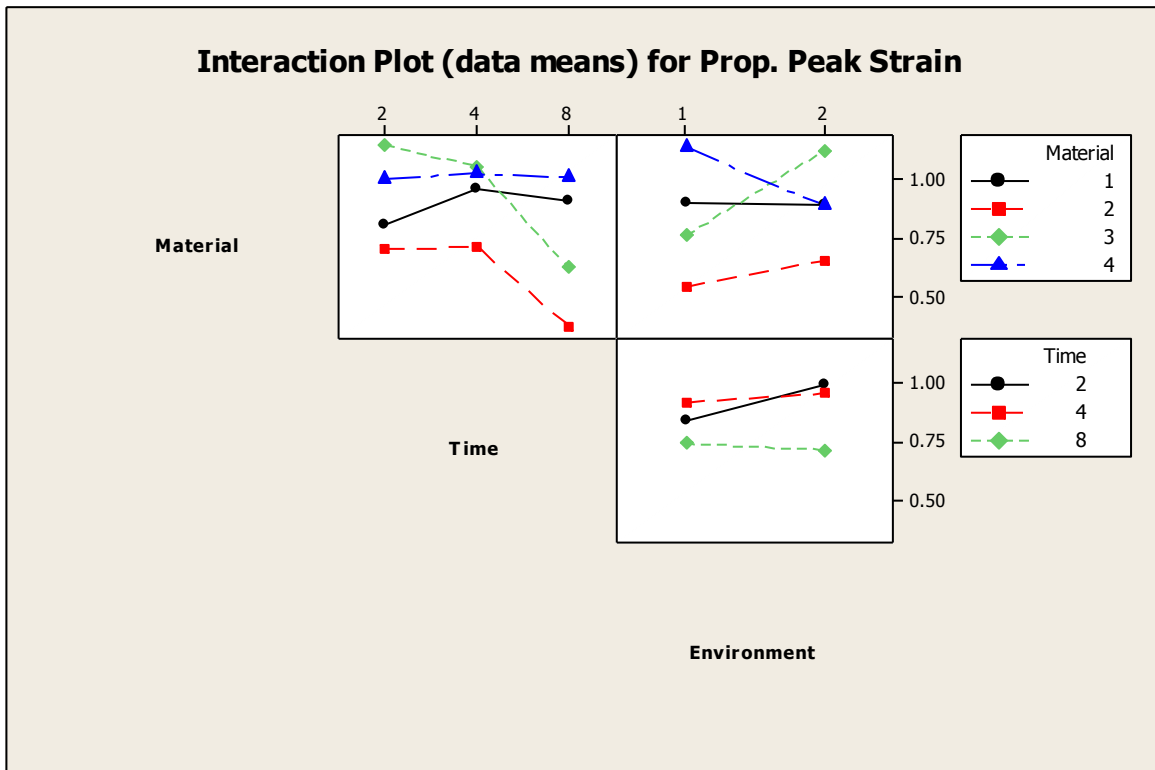


Figure 74: Interaction plot for proportional peak strain, materials 1-4

ANOVA: Proportion of Peak Strain from Baseline versus Material, Degradation Time and Degradation Environment (materials 1-4)

Factor	Type	Levels	Values
Material	fixed	4	1, 2, 3, 4
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Prop. Peak Strain

Source	DF	SS	MS	F	P
Material	3	1.83268	0.61089	8.71	0.000
Time	2	0.63794	0.31897	4.55	0.015
Environment	1	0.05416	0.05416	0.77	0.383
Material*Time	6	0.82652	0.13775	1.96	0.087
Material*Environment	3	0.88516	0.29505	4.21	0.010
Time*Environment	2	0.10588	0.05294	0.76	0.475
Error	54	3.78613	0.07011		
Total	71	8.12847			

S = 0.264790 R-Sq = 53.42% R-Sq(adj) = 38.76%

Expected Mean Square for

Source	Variance component	Error term	Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 24 Q[2]
3 Environment		7	(7) + 36 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 12 Q[6]
7 Error	0.07011		(7)

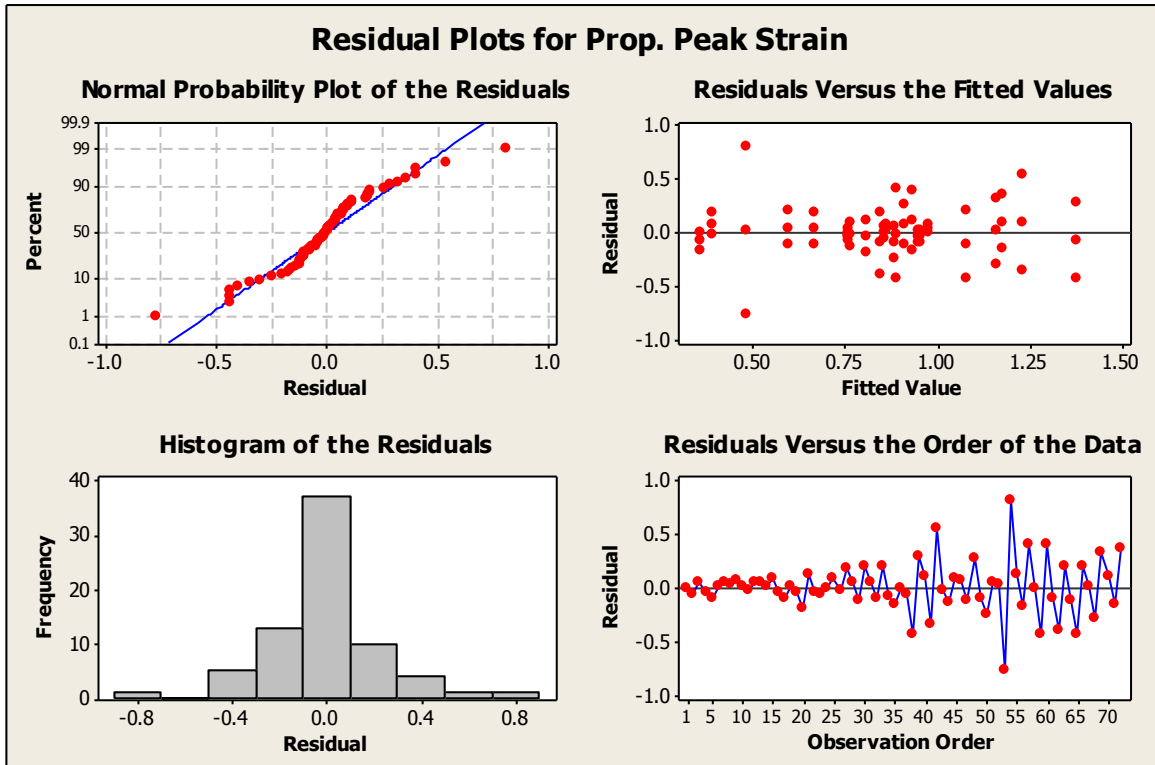


Figure 75: Residual plots for proportional peak strain, materials 1-4

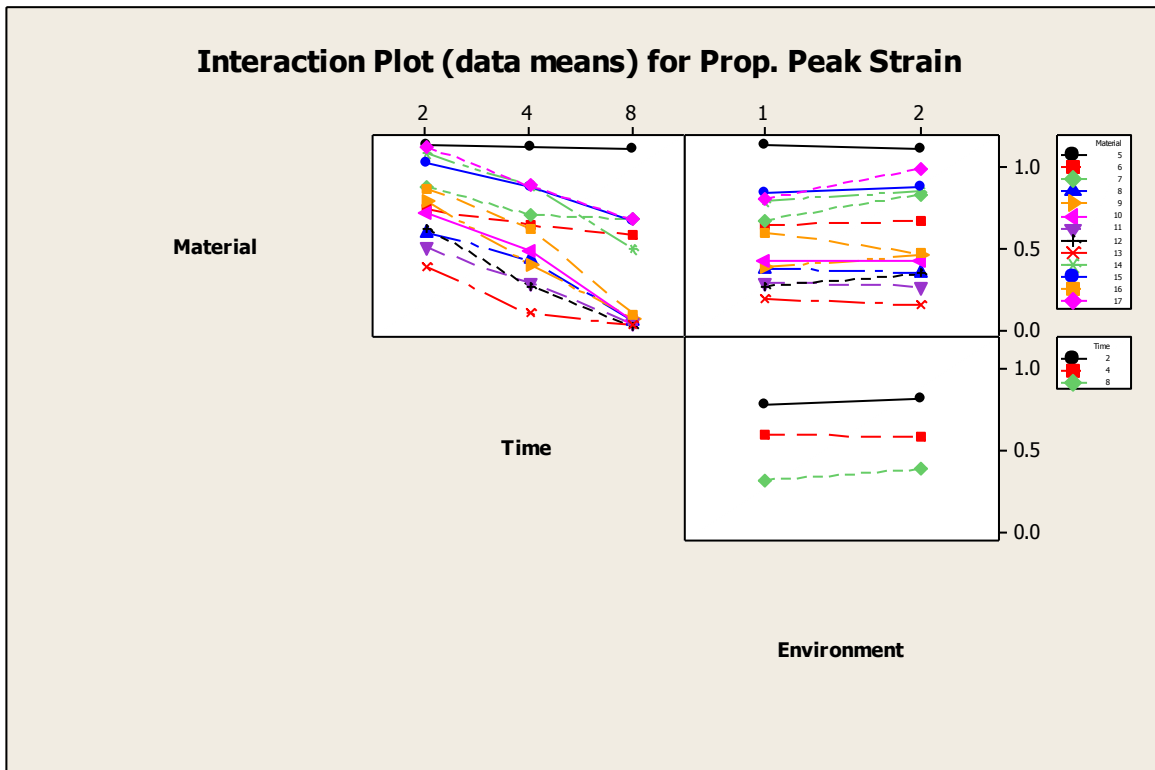


Figure 76: Interaction plot for proportional peak strain, materials 5-17

ANOVA: Proportion of Peak Strain from Baseline versus Material, Degradation Time and Degradation Environment (materials 5-17)

Factor	Type	Levels	Values
Material	fixed	13	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Prop. Peak Strain

Source	DF	SS	MS	F	P
Material	12	17.95527	1.49627	86.03	0.000
Time	2	8.01680	4.00840	230.48	0.000
Environment	1	0.04938	0.04938	2.84	0.094
Material*Time	24	2.16749	0.09031	5.19	0.000
Material*Environment	12	0.38323	0.03194	1.84	0.045
Time*Environment	2	0.08358	0.04179	2.40	0.093
Error	180	3.13050	0.01739		
Total	233	31.78626			

S = 0.131878 R-Sq = 90.15% R-Sq(adj) = 87.25%

Expected Mean
Square for

Source	Variance component	Error term	Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 78 Q[2]
3 Environment		7	(7) + 117 Q[3]
4 Material*Time		7	(7) + 6 Q[4]
5 Material*Environment		7	(7) + 9 Q[5]
6 Time*Environment		7	(7) + 39 Q[6]
7 Error	0.01739		(7)

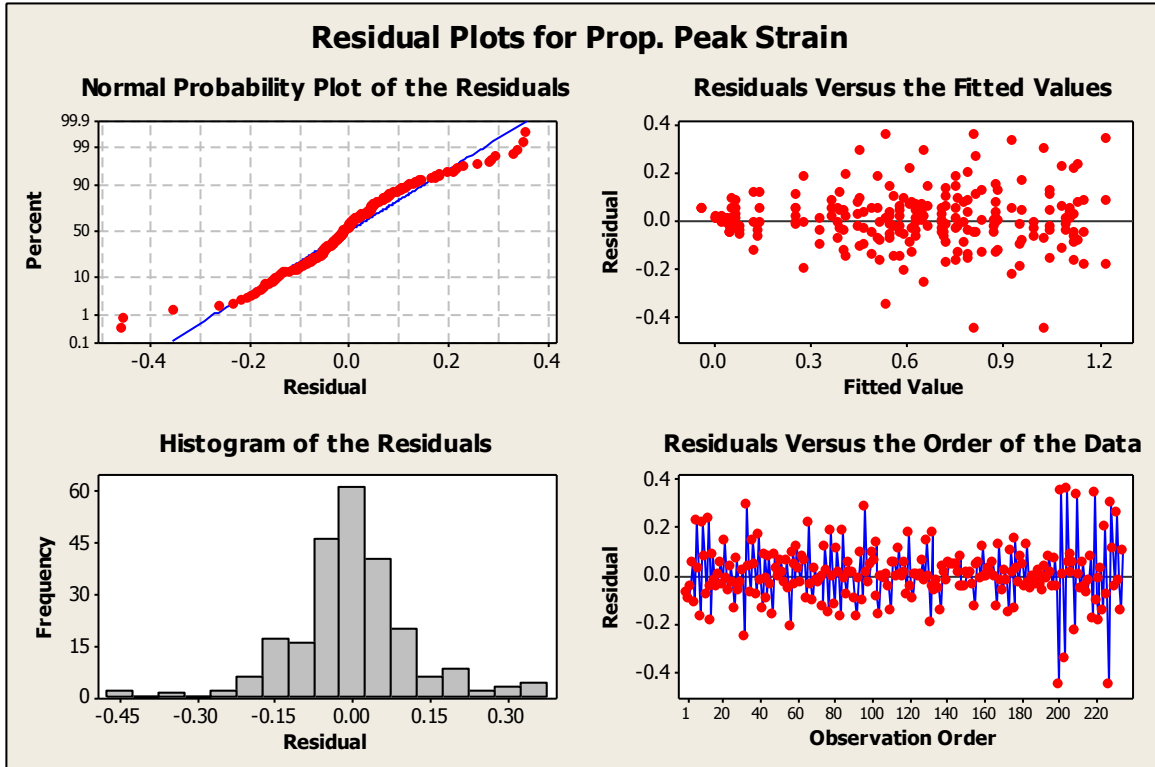


Figure 77: Residual plots for proportional peak strain, materials 5-17

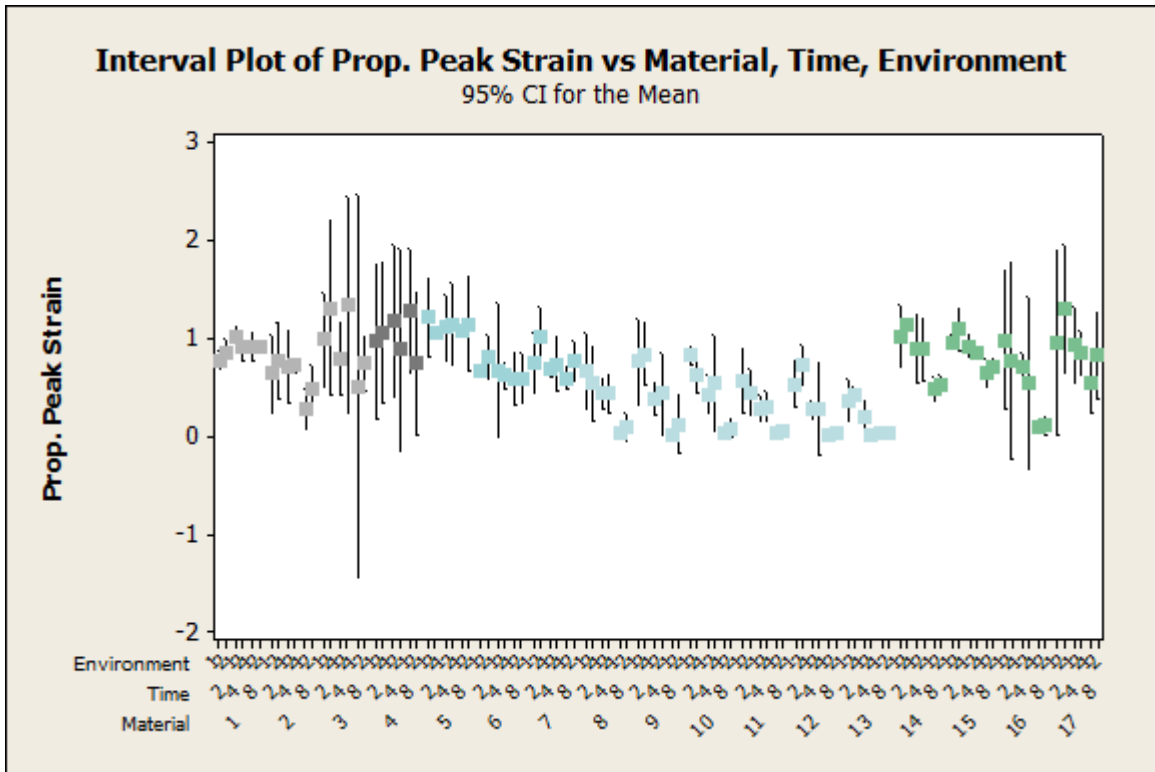


Figure 78: Interval plot of proportional peak strain vs material, degradation time and degradation environment

Individual Material ANOVAs of Proportional Peak Strain by Degradation Time:

One-way ANOVA: Prop. Peak Strain versus Time for Material 1

Source	DF	SS	MS	F	P
Time	2	0.07334	0.03667	10.18	0.002
Error	15	0.05404	0.00360		
Total	17	0.12739			

S = 0.06002 R-Sq = 57.58% R-Sq(adj) = 51.92%

Level	N	Mean	StDev
2	6	0.8097	0.0628
4	6	0.9638	0.0729
8	6	0.9097	0.0395

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 0.0600

Tukey 95% Simultaneous Confidence Intervals

All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	0.06415	0.15409	0.24402
8	0.01012	0.10005	0.18998

-----+-----+-----+-----+-----
 (-----*-----)
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.10 0.00 0.10 0.20

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.14396	-0.05403	0.03590

-----+-----+-----+-----+-----
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.10 0.00 0.10 0.20

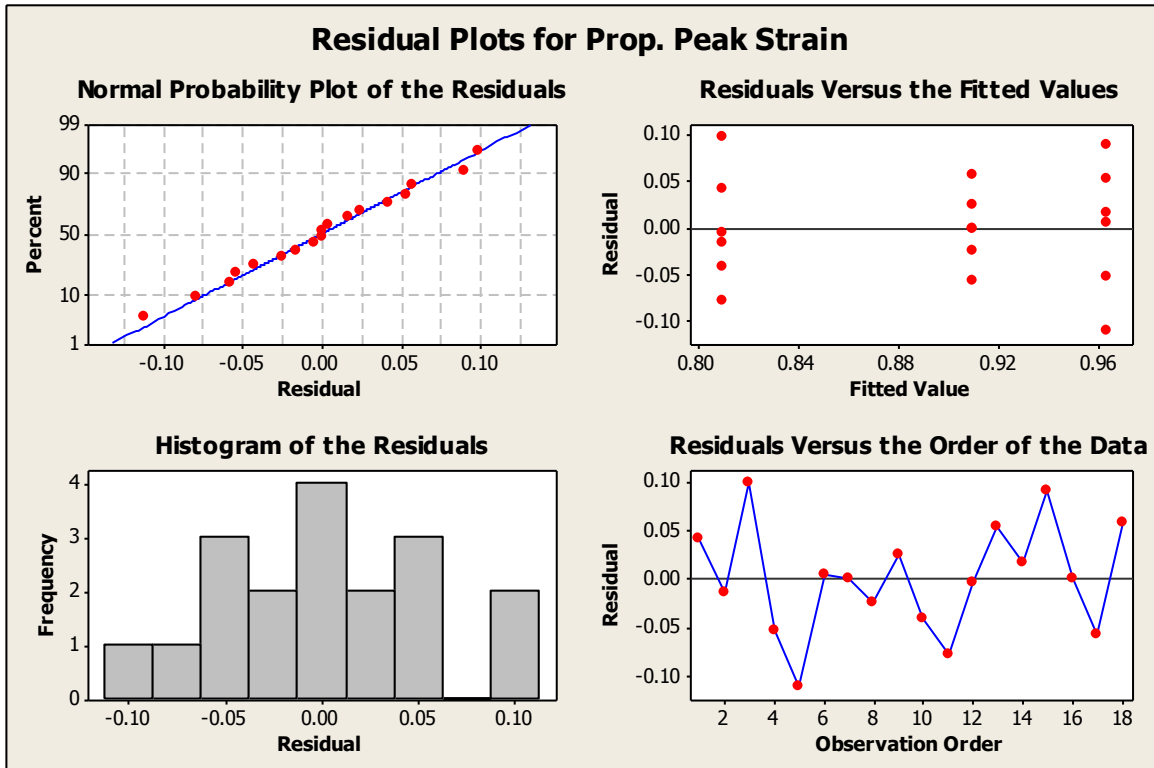
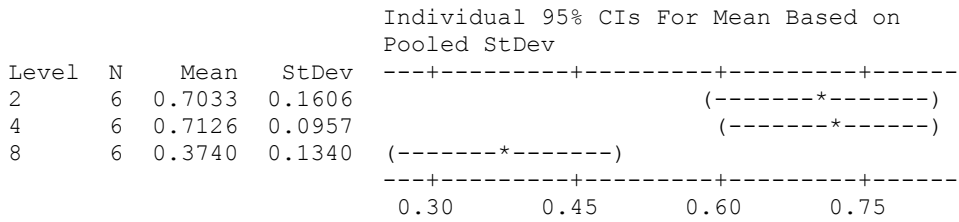


Figure 79: Residual plots for proportional peak strain, material 1

One-way ANOVA: Prop. Peak Strain versus Time for Material 2

Source	DF	SS	MS	F	P
Time	2	0.4464	0.2232	12.66	0.001
Error	15	0.2645	0.0176		
Total	17	0.7108			

S = 0.1328 R-Sq = 62.79% R-Sq(adj) = 57.83%

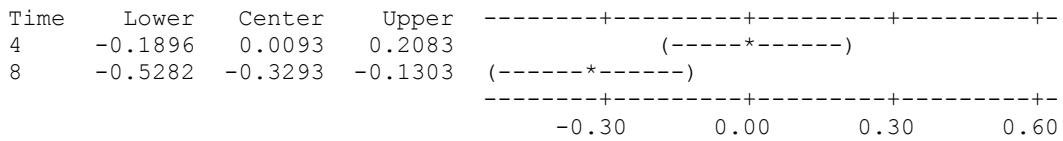


Pooled StDev = 0.1328

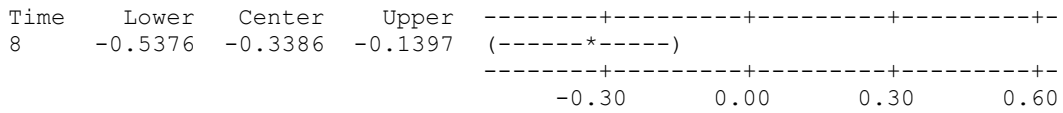
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



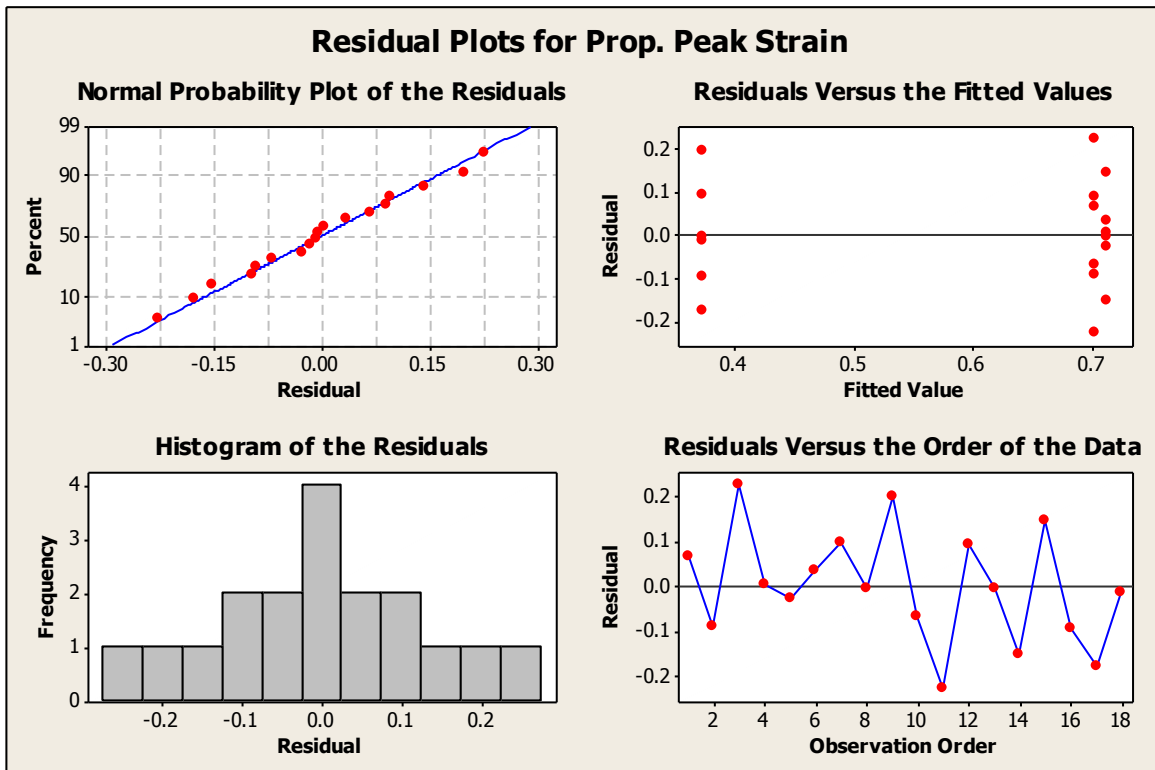


Figure 80: Residual plots for proportional peak strain, material 2

One-way ANOVA: Prop. Peak Strain versus Time for Material 3

Source	DF	SS	MS	F	P
Time	2	0.944	0.472	2.60	0.107
Error	15	2.719	0.181		
Total	17	3.663			

S = 0.4258 R-Sq = 25.76% R-Sq(adj) = 15.86%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
2	6	1.1470	0.3127	(-----*-----)
4	6	1.0605	0.4192	(-----*-----)
8	6	0.6239	0.5200	(-----*-----)

Pooled StDev = 0.4258

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.7245	-0.0866	0.5514
8	-1.1611	-0.5231	0.1148

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-1.0745	-0.4366	0.2014

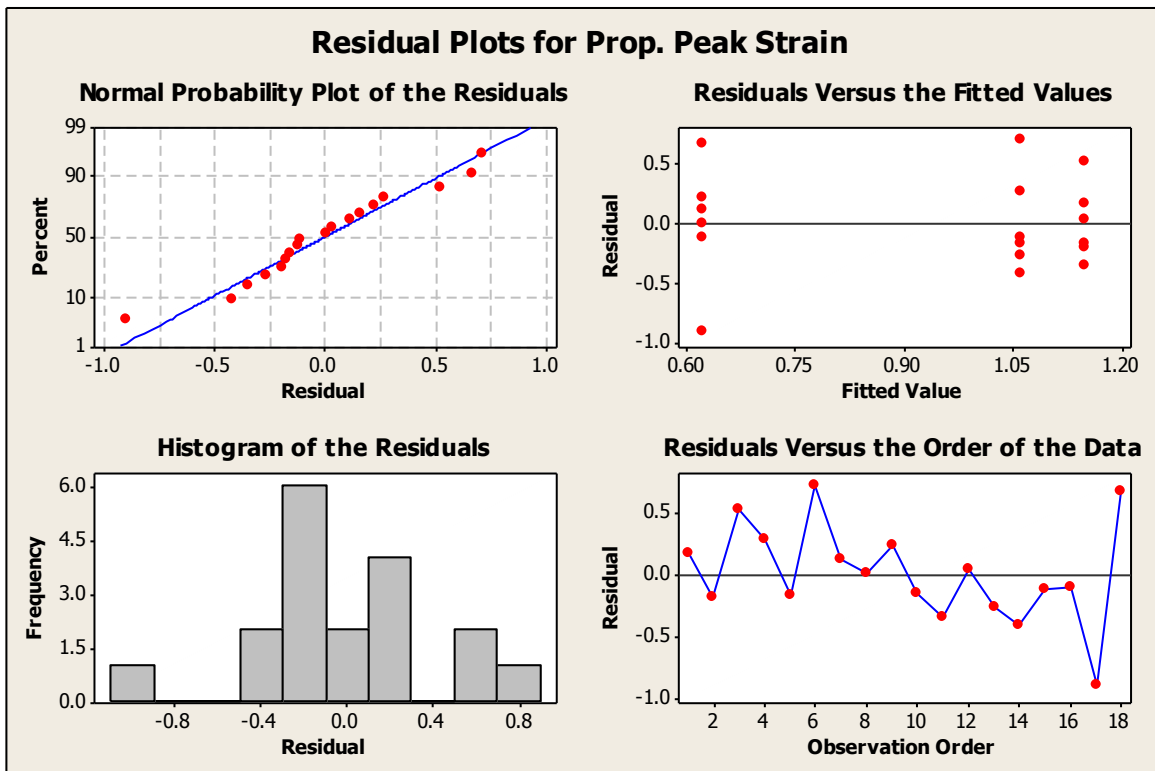


Figure 81: Residual plots for proportional peak strain, material 3

One-way ANOVA: Prop. Peak Strain versus Time for Material 4

Source	DF	SS	MS	F	P
Time	2	0.001	0.001	0.00	0.995
Error	15	1.793	0.120		
Total	17	1.795			

S = 0.3458 R-Sq = 0.07% R-Sq(adj) = 0.00%

Individual 95% CIs For Mean Based on

Level	N	Mean	StDev	Pooled StDev
2	6	1.0073	0.2751	(-----*-----)
4	6	1.0268	0.3677	(-----*-----)
8	6	1.0135	0.3844	(-----*-----)

0.80 0.96 1.12 1.28

Pooled StDev = 0.3458

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper	
4	-0.4986	0.0195	0.5376	(-----*-----)
8	-0.5119	0.0062	0.5243	(-----*-----)

-0.30 0.00 0.30 0.60

Time = 4 subtracted from:

Time	Lower	Center	Upper	
8	-0.5314	-0.0133	0.5048	(-----*-----)

-0.30 0.00 0.30 0.60

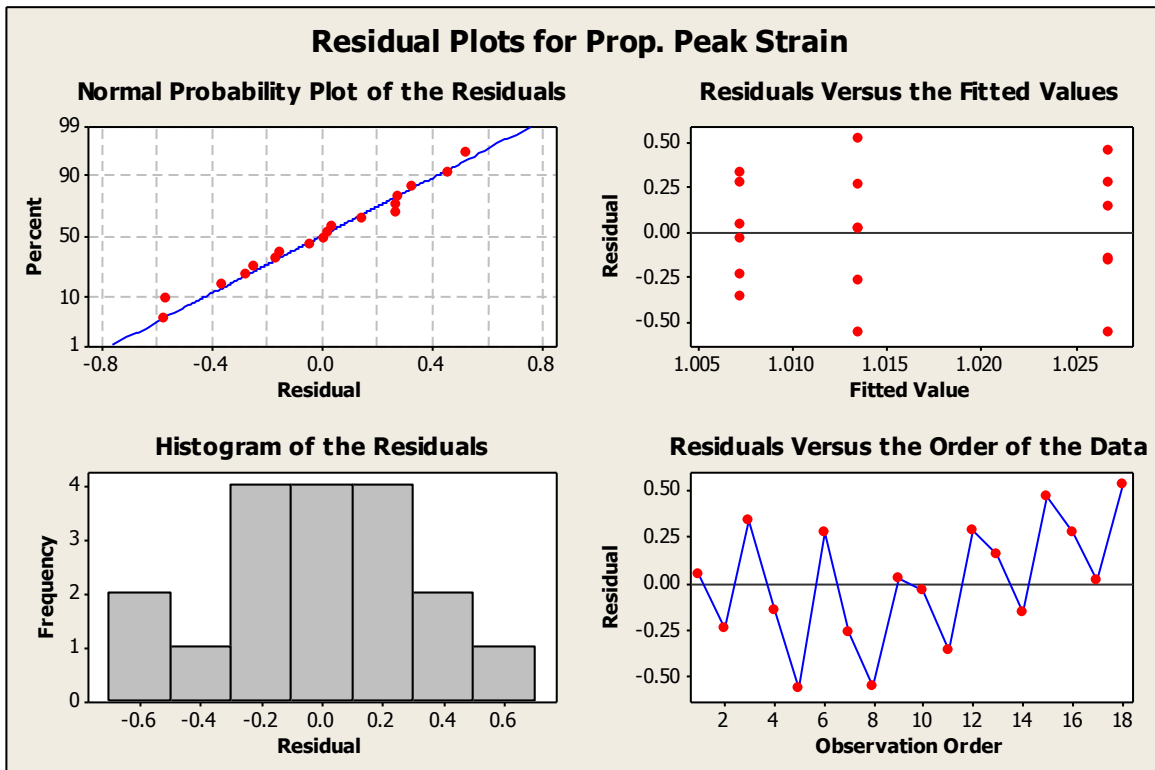


Figure 82: Residual plots for proportional peak strain, material 4

One-way ANOVA: Prop. Peak Strain versus Time for Material 5

Source	DF	SS	MS	F	P
Time	2	0.0011	0.0006	0.03	0.969
Error	15	0.2685	0.0179		
Total	17	0.2696			

S = 0.1338 R-Sq = 0.41% R-Sq(adj) = 0.00%

Level	N	Mean	StDev
2	6	1.1301	0.1338
4	6	1.1218	0.1390
8	6	1.1109	0.1283

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 0.1338

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.2088	-0.0084	0.1921
8	-0.2197	-0.0192	0.1812

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.2113	-0.0109	0.1896

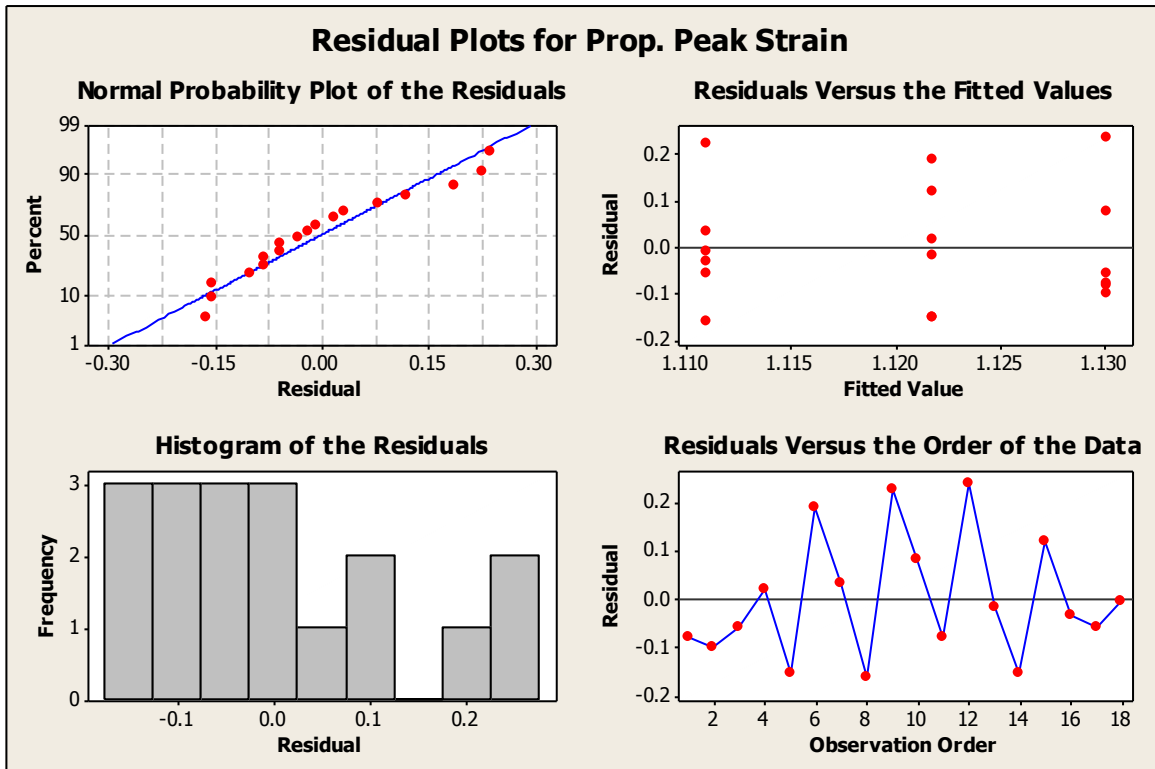
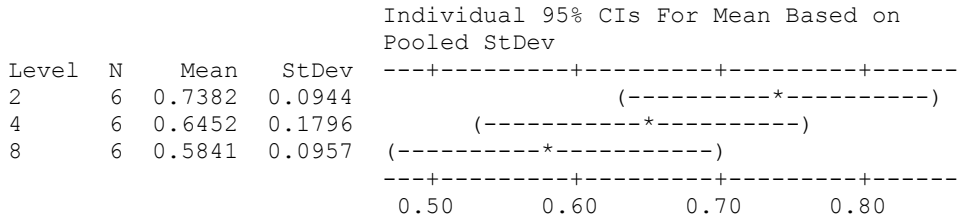


Figure 83: Residual plots for proportional peak strain, material 5

One-way ANOVA: Prop. Peak Strain versus Time for Material 6

Source	DF	SS	MS	F	P
Time	2	0.0722	0.0361	2.15	0.151
Error	15	0.2516	0.0168		
Total	17	0.3238			

S = 0.1295 R-Sq = 22.31% R-Sq(adj) = 11.95%

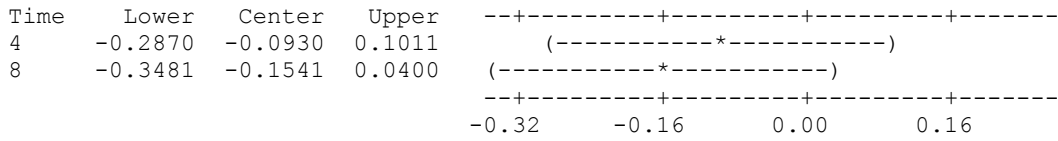


Pooled StDev = 0.1295

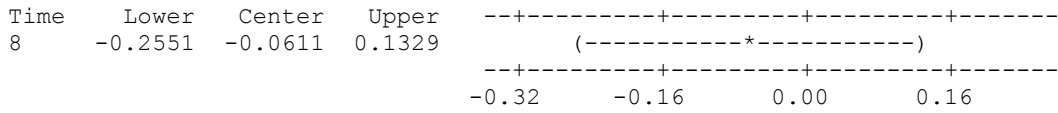
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



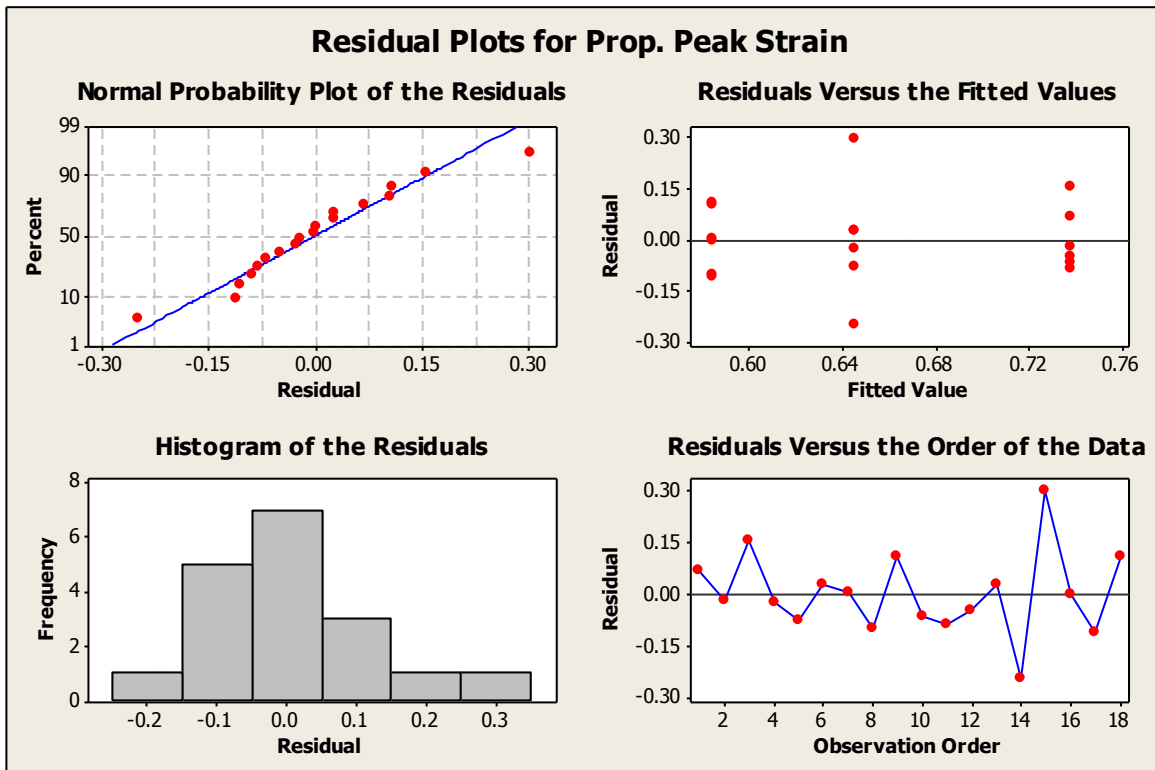


Figure 84: Residual plots for proportional peak strain, material 6

One-way ANOVA: Prop. Peak Strain versus Time for Material 7

Source	DF	SS	MS	F	P
Time	2	0.1382	0.0691	4.09	0.038
Error	15	0.2532	0.0169		
Total	17	0.3915			

S = 0.1299 R-Sq = 35.31% R-Sq(adj) = 26.68%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
2	6	0.8778	0.1773	(-----*-----)
4	6	0.7078	0.0800	(-----*-----)
8	6	0.6792	0.1131	(-----*-----)

-----+-----+-----+-----
0.60 0.72 0.84 0.96

Pooled StDev = 0.1299

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.3646	-0.1700	0.0247
8	-0.3932	-0.1985	-0.0038

-----+-----+-----+-----+-----
 (-----*-----)
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.32 -0.16 0.00 0.16

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.2232	-0.0286	0.1661

-----+-----+-----+-----+-----
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.32 -0.16 0.00 0.16

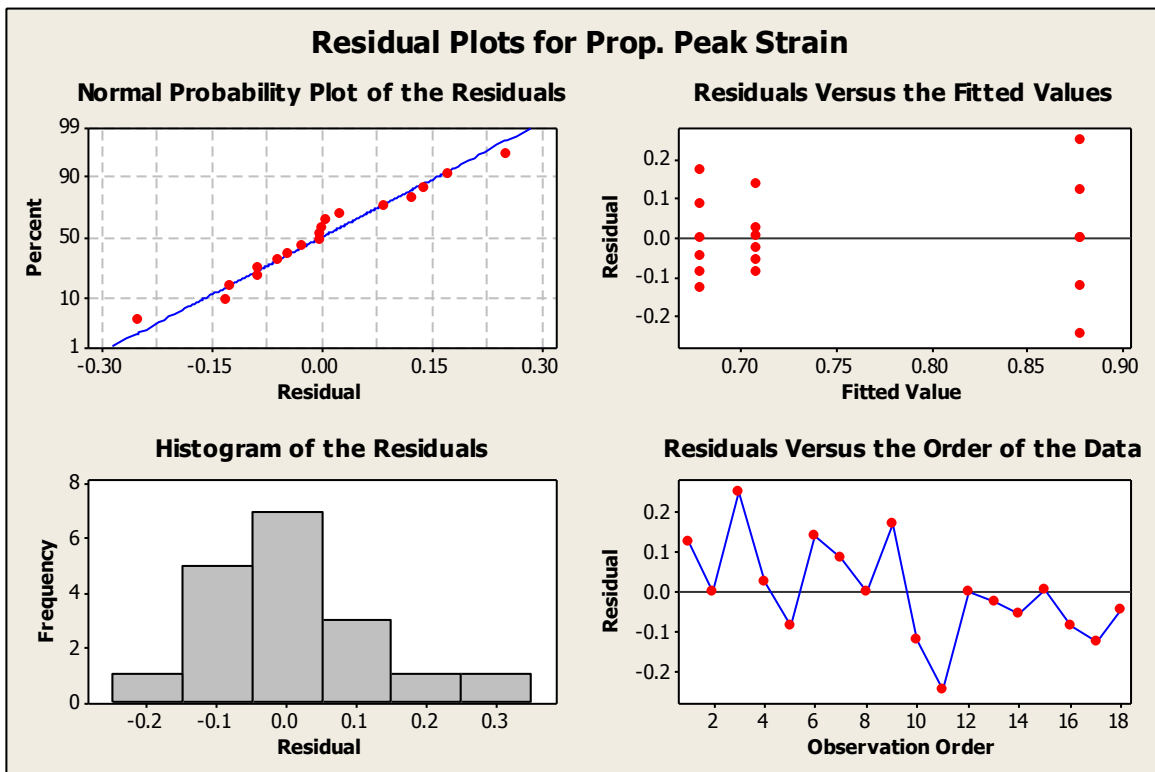


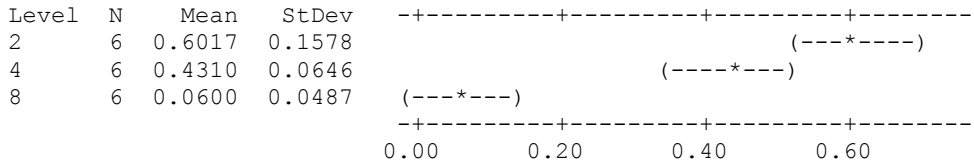
Figure 85: Residual plots for proportional peak strain, material 7

One-way ANOVA: Prop. Peak Strain versus Time for Material 8

Source	DF	SS	MS	F	P
Time	2	0.9204	0.4602	43.89	0.000
Error	15	0.1573	0.0105		
Total	17	1.0777			

S = 0.1024 R-Sq = 85.40% R-Sq(adj) = 83.46%

Individual 95% CIs For Mean Based on Pooled StDev

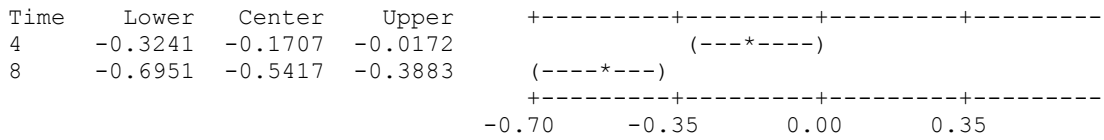


Pooled StDev = 0.1024

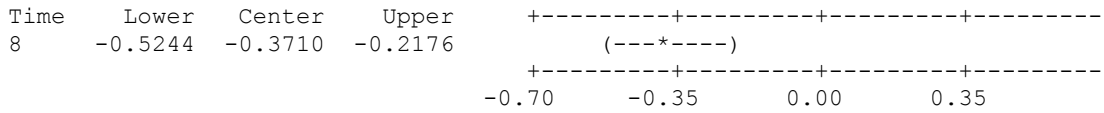
Tukey 95% Simultaneous Confidence Intervals
 All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



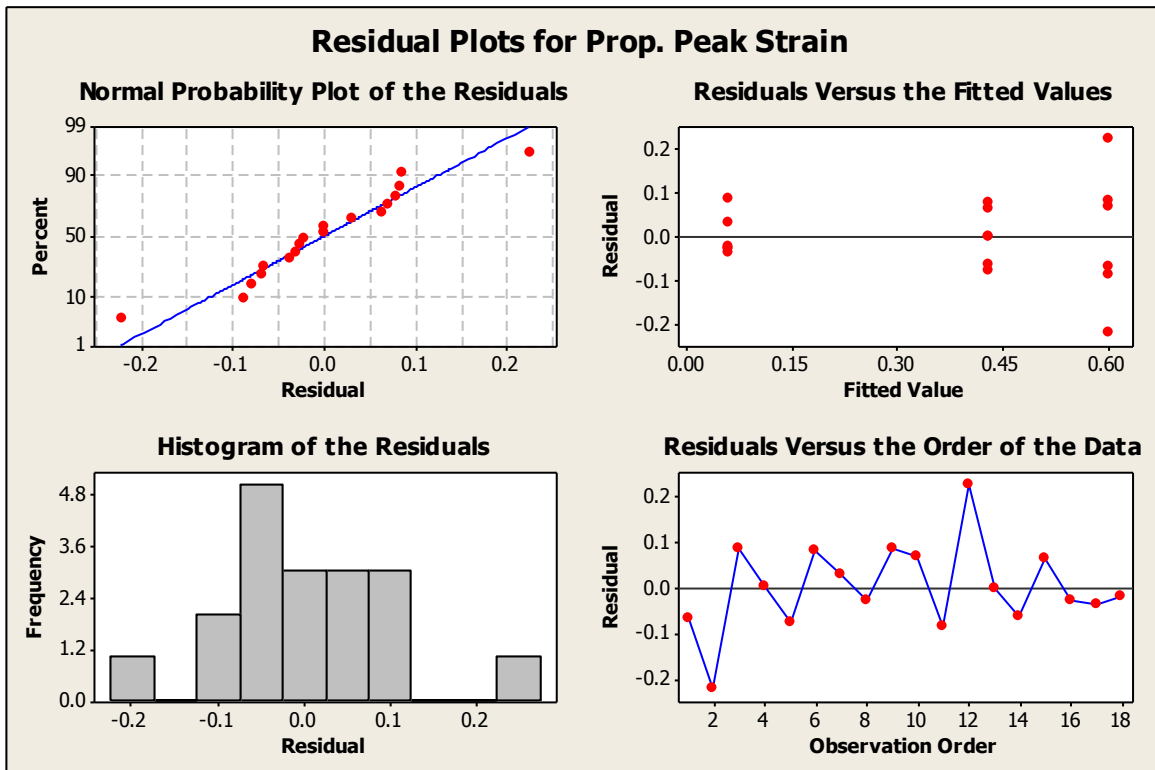
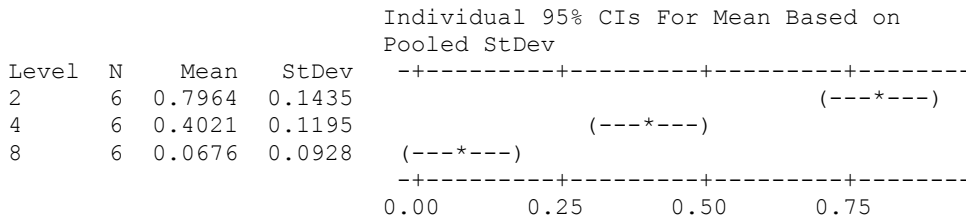


Figure 86: Residual plots for proportional peak strain, material 8

One-way ANOVA: Prop. Peak Strain versus Time for Material 9

Source	DF	SS	MS	F	P
Time	2	1.5972	0.7986	55.09	0.000
Error	15	0.2174	0.0145		
Total	17	1.8146			

S = 0.1204 R-Sq = 88.02% R-Sq(adj) = 86.42%



Pooled StDev = 0.1204

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.5747	-0.3943	-0.2139
8	-0.9092	-0.7288	-0.5484

(---*---)
(----*---)

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.5150	-0.3346	-0.1542

(----*---)

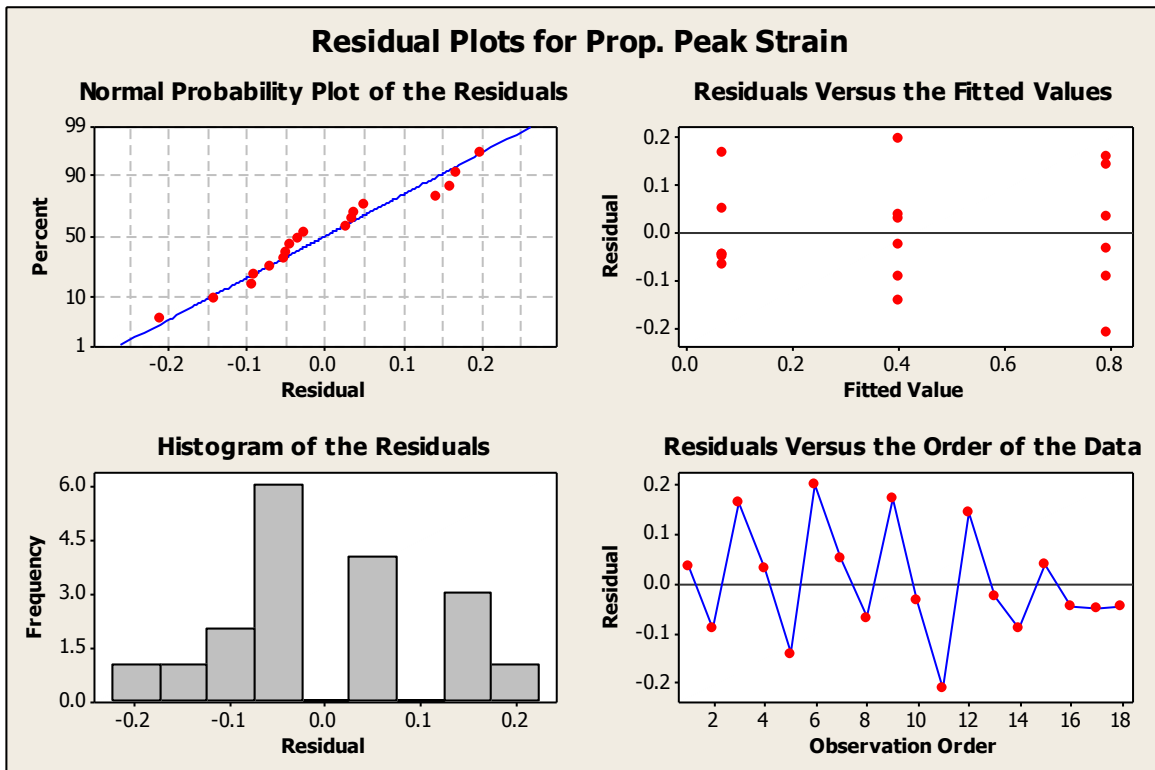


Figure 87: Residual plots for proportional peak strain, material 9

One-way ANOVA: Prop. Peak Strain versus Time for Material 10

Source	DF	SS	MS	F	P
Time	2	1.3748	0.6874	54.83	0.000
Error	15	0.1881	0.0125		
Total	17	1.5629			

S = 0.1120 R-Sq = 87.97% R-Sq(adj) = 86.36%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
2	6	0.7238	0.1179
4	6	0.4877	0.1498
8	6	0.0563	0.0355

---+-----+-----+-----+-----
 (---*---) (---*---) (---*---)
 ---+-----+-----+-----+-----
 0.00 0.25 0.50 0.75

Pooled StDev = 0.1120

Tukey 95% Simultaneous Confidence Intervals
 All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.4039	-0.2361	-0.0684
8	-0.8353	-0.6675	-0.4997

---+-----+-----+-----+-----
 (---*---) (---*---)
 ---+-----+-----+-----+-----
 -0.80 -0.40 -0.00 0.40

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.5991	-0.4314	-0.2636

---+-----+-----+-----+-----
 (---*---)
 ---+-----+-----+-----+-----
 -0.80 -0.40 -0.00 0.40

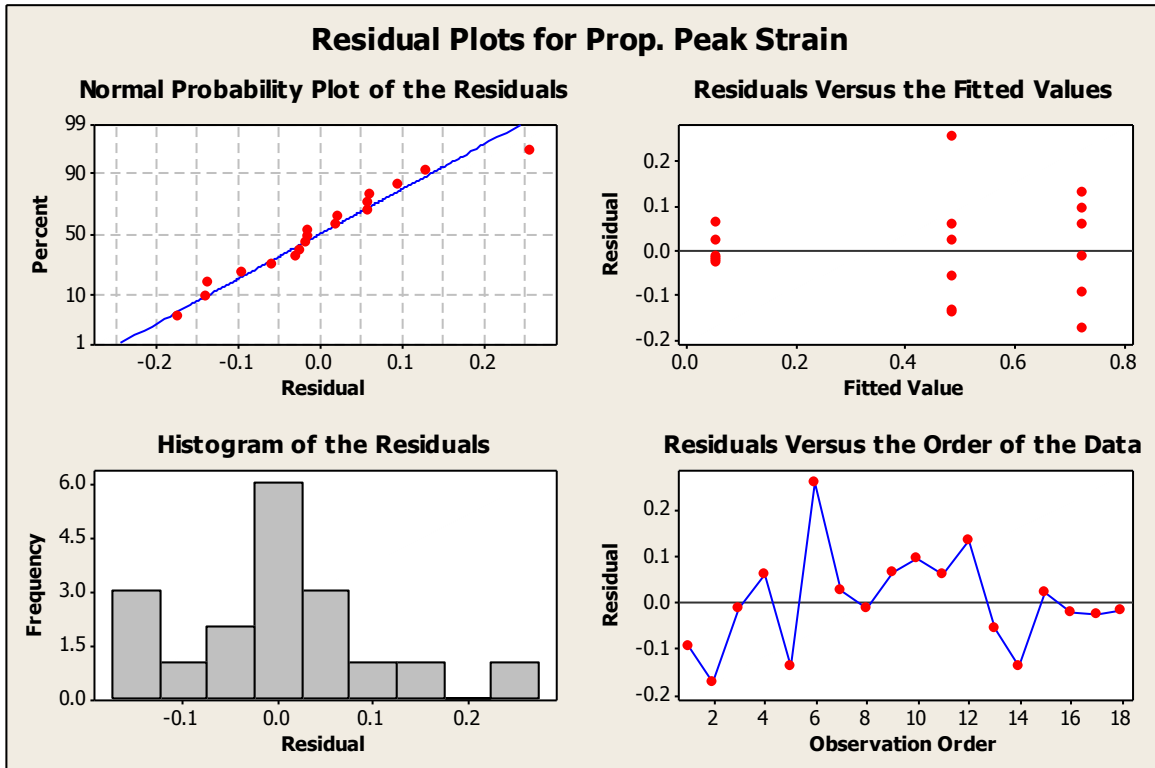


Figure 88: Residual plots for proportional peak strain, material 10

One-way ANOVA: Prop. Peak Strain versus Time for Material 11

Source	DF	SS	MS	F	P
Time	2	0.65675	0.32837	56.94	0.000
Error	15	0.08650	0.00577		
Total	17	0.74325			

S = 0.07594 R-Sq = 88.36% R-Sq(adj) = 86.81%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
2	6	0.50537	0.12037
4	6	0.29318	0.05195
8	6	0.03814	0.01064

0.00 0.15 0.30 0.45

Pooled StDev = 0.07594

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.32597	-0.21219	-0.09841
8	-0.58101	-0.46723	-0.35345

(---*---)

(---*---)

-----+-----+-----+-----+-----

-0.50 -0.25 0.00 0.25

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.36882	-0.25504	-0.14126

(---*---)

-----+-----+-----+-----+-----

-0.50 -0.25 0.00 0.25

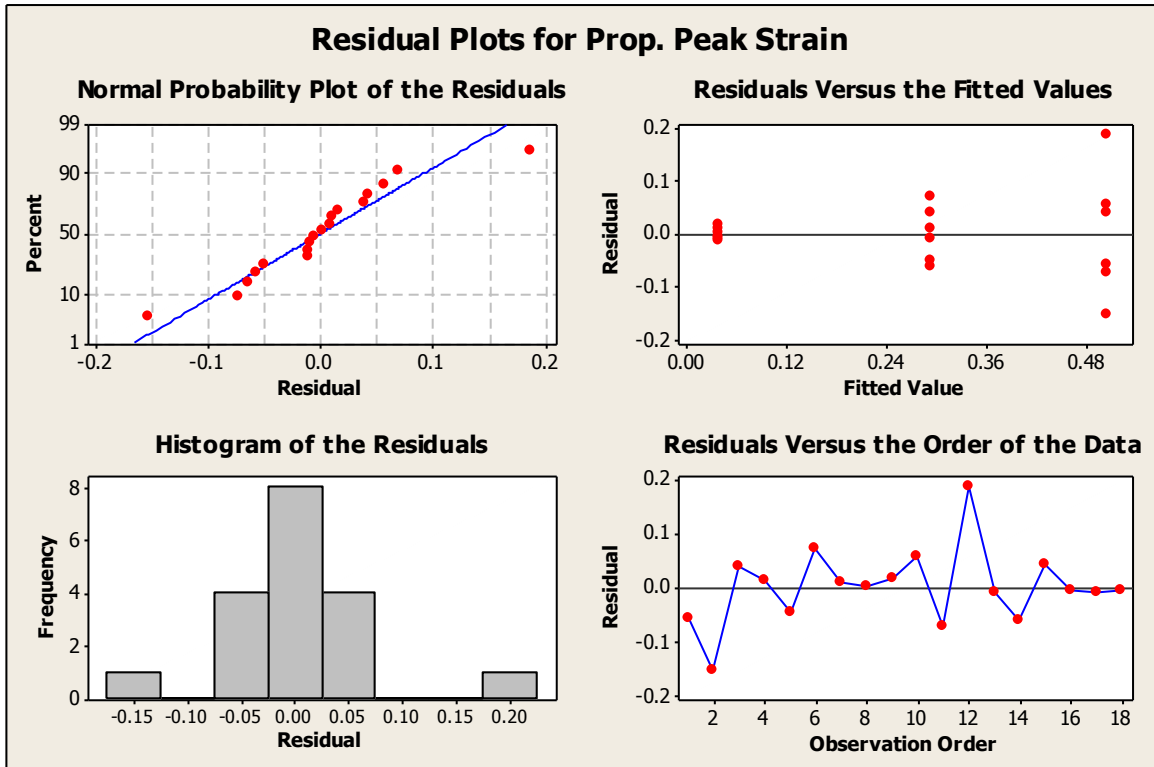


Figure 89: Residual plots for proportional peak strain, material 11

One-way ANOVA: Prop. Peak Strain versus Time for Material 12

Source	DF	SS	MS	F	P
Time	2	1.0980	0.5490	48.91	0.000
Error	15	0.1684	0.0112		
Total	17	1.2664			

S = 0.1059 R-Sq = 86.71% R-Sq(adj) = 84.93%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev

-----+-----+-----+-----+-----

2	6	0.6255	0.1357	(---*---)
4	6	0.2682	0.1222	(---*---)
8	6	0.0240	0.0186	(---*---)

-----+-----+-----+-----+-----
0.00 0.20 0.40 0.60

Pooled StDev = 0.1059

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----
4	-0.5160	-0.3573	-0.1985	(---*---)
8	-0.7602	-0.6015	-0.4427	(---*---)

-----+-----+-----+-----+-----
-0.60 -0.30 0.00 0.30

Time = 4 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----
8	-0.4029	-0.2442	-0.0855	(---*---)

-----+-----+-----+-----+-----
-0.60 -0.30 0.00 0.30

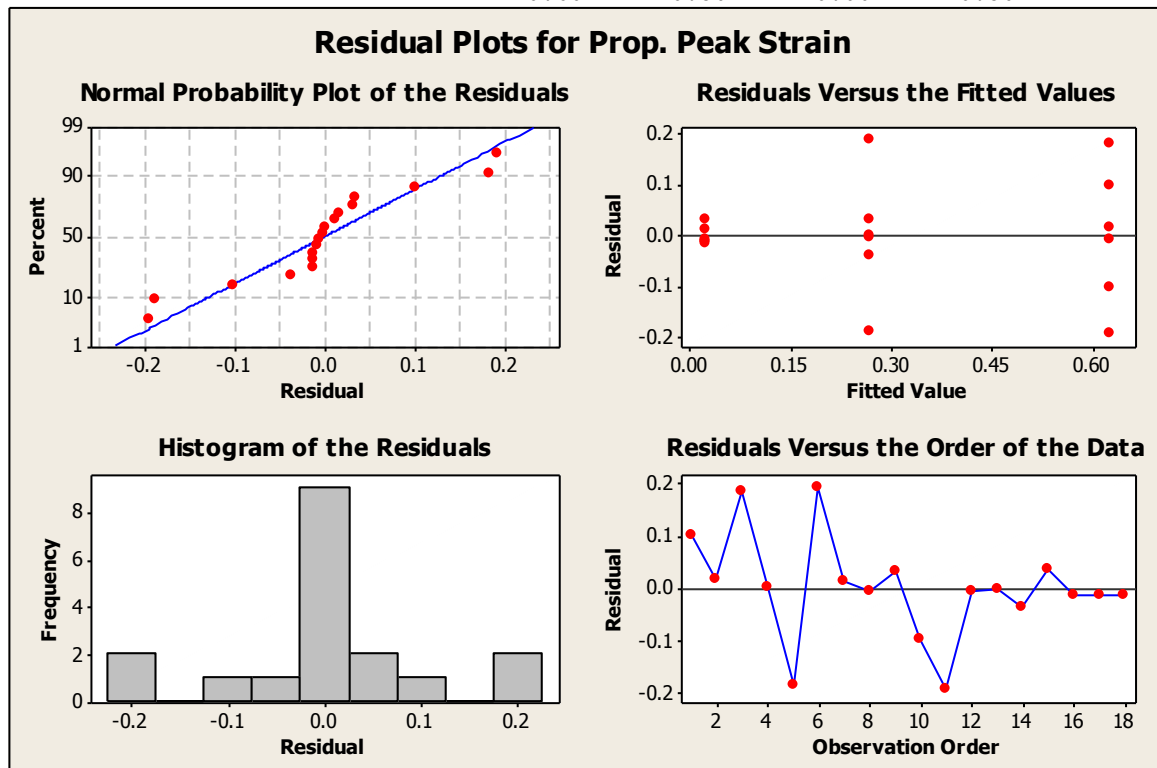
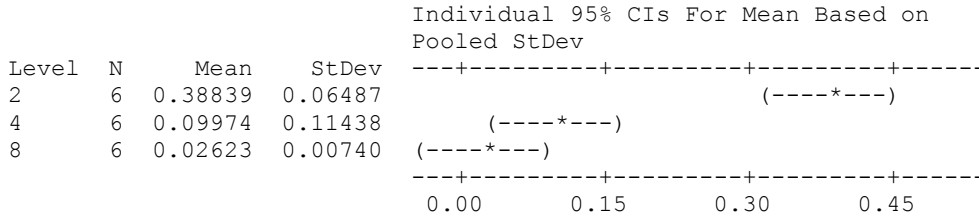


Figure 90: Residual plots for proportional peak strain, material 12

One-way ANOVA: Prop. Peak Strain versus Time for Material 13

Source	DF	SS	MS	F	P
Time	2	0.43975	0.21988	38.03	0.000
Error	15	0.08673	0.00578		
Total	17	0.52648			

S = 0.07604 R-Sq = 83.53% R-Sq(adj) = 81.33%

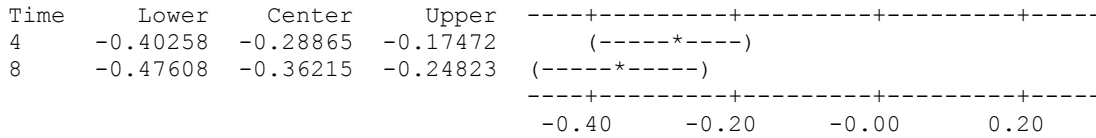


Pooled StDev = 0.07604

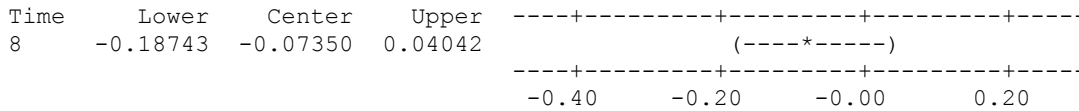
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



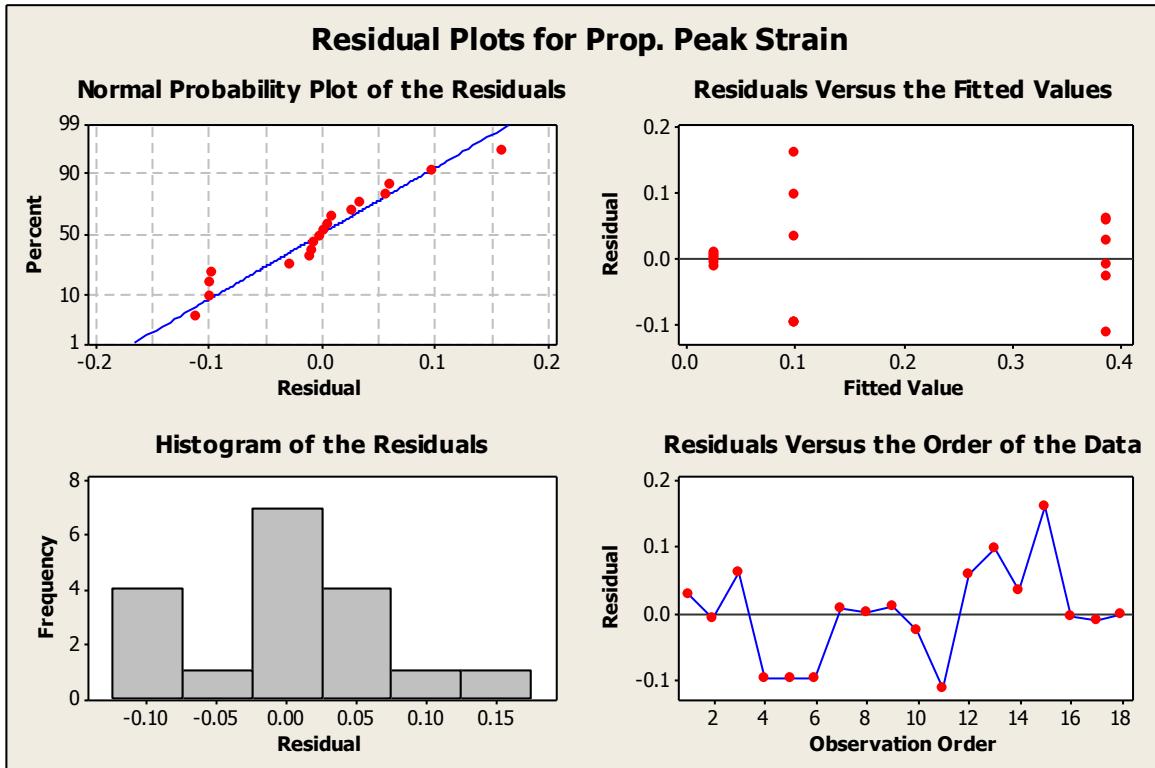
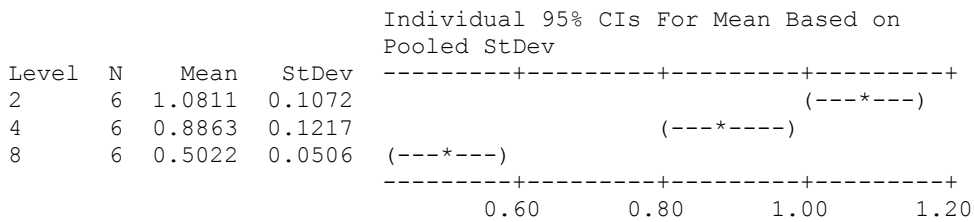


Figure 91: Residual plots for proportional peak strain, material 13

One-way ANOVA: Prop. Peak Strain versus Time for Material 14

Source	DF	SS	MS	F	P
Time	2	1.04120	0.52060	54.14	0.000
Error	15	0.14423	0.00962		
Total	17	1.18543			

S = 0.09806 R-Sq = 87.83% R-Sq(adj) = 86.21%



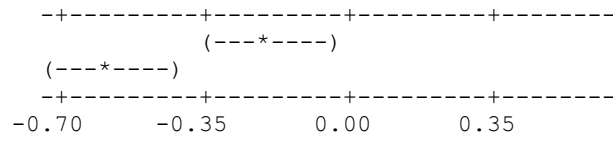
Pooled StDev = 0.0981

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.34171	-0.19479	-0.04787
8	-0.72581	-0.57890	-0.43198



Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.53103	-0.38411	-0.23719

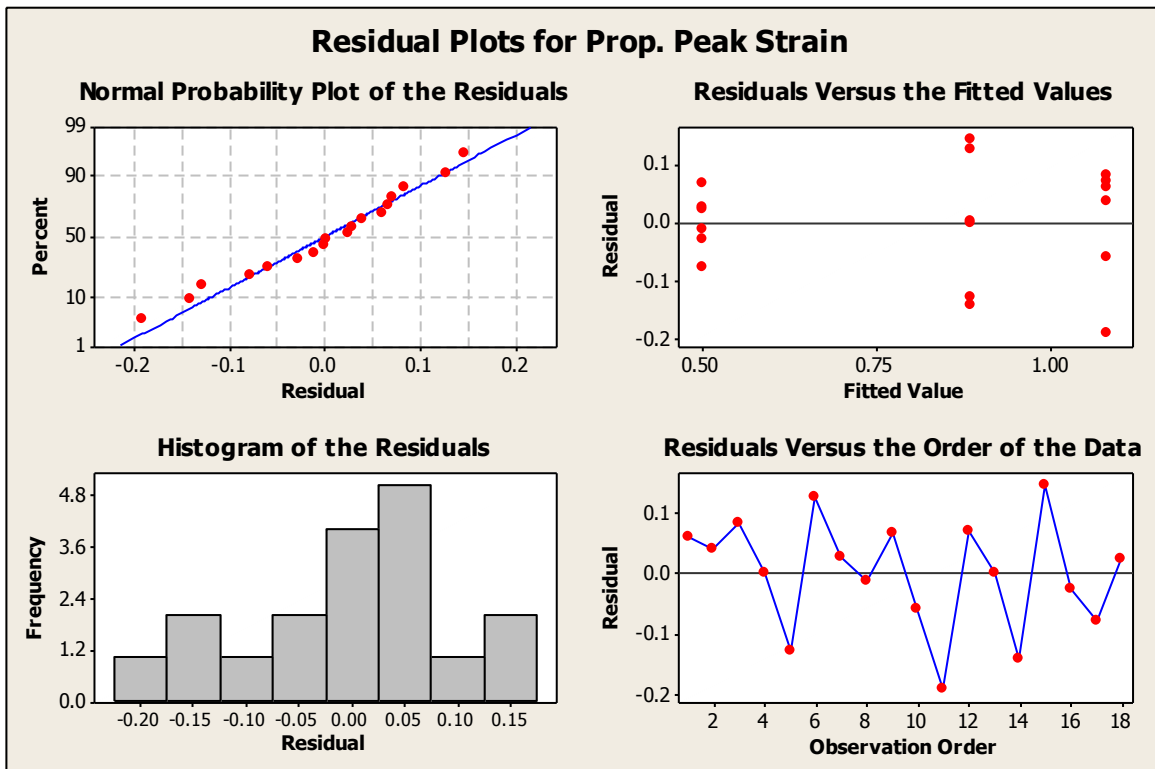
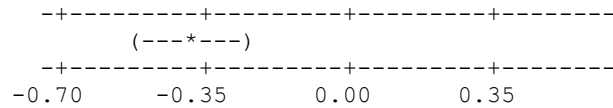
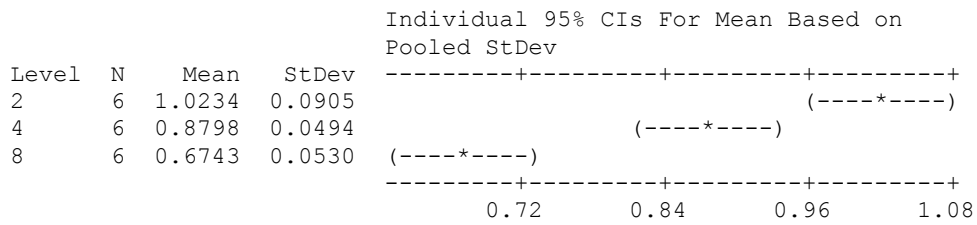


Figure 92: Residual plots for proportional peak strain, material 14

One-way ANOVA: Prop. Peak Strain versus Time for Material 15

Source	DF	SS	MS	F	P
Time	2	0.36954	0.18477	41.21	0.000
Error	15	0.06725	0.00448		
Total	17	0.43679			

S = 0.06696 R-Sq = 84.60% R-Sq(adj) = 82.55%

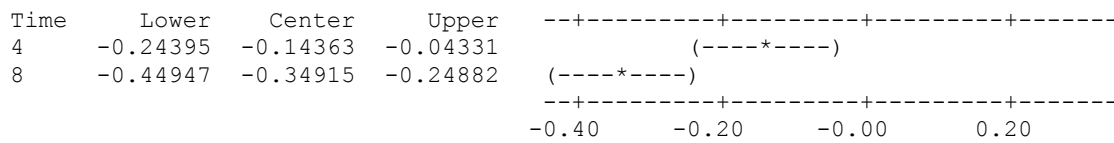


Pooled StDev = 0.0670

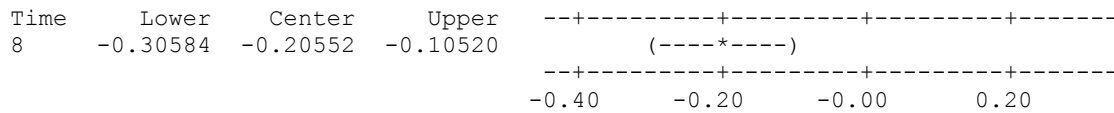
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



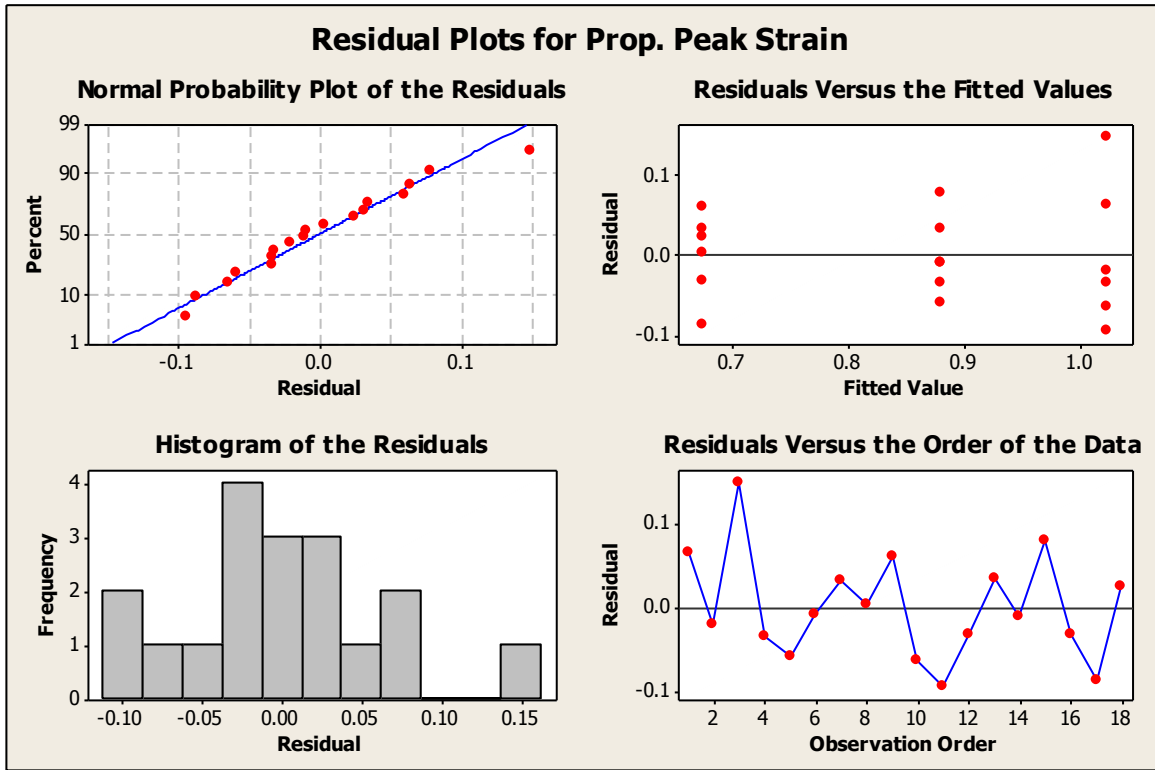
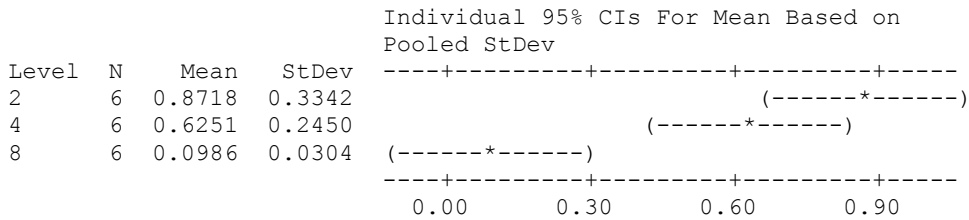


Figure 93: Residual plots for proportional peak strain, material 15

One-way ANOVA: Prop. Peak Strain versus Time for Material 16

Source	DF	SS	MS	F	P
Time	2	1.8719	0.9360	16.26	0.000
Error	15	0.8633	0.0576		
Total	17	2.7352			

S = 0.2399 R-Sq = 68.44% R-Sq(adj) = 64.23%



Pooled StDev = 0.2399

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.6061	-0.2467	0.1127
8	-1.1327	-0.7732	-0.4138

-----+-----+-----+-----+-----+
 (-----*-----)
 (-----*-----)
 -----+-----+-----+-----+-----+

-0.60 0.00 0.60 1.20

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.8859	-0.5265	-0.1671

-----+-----+-----+-----+-----+
 (-----*-----)
 -----+-----+-----+-----+-----+

-0.60 0.00 0.60 1.20

Residual Plots for Prop. Peak Strain

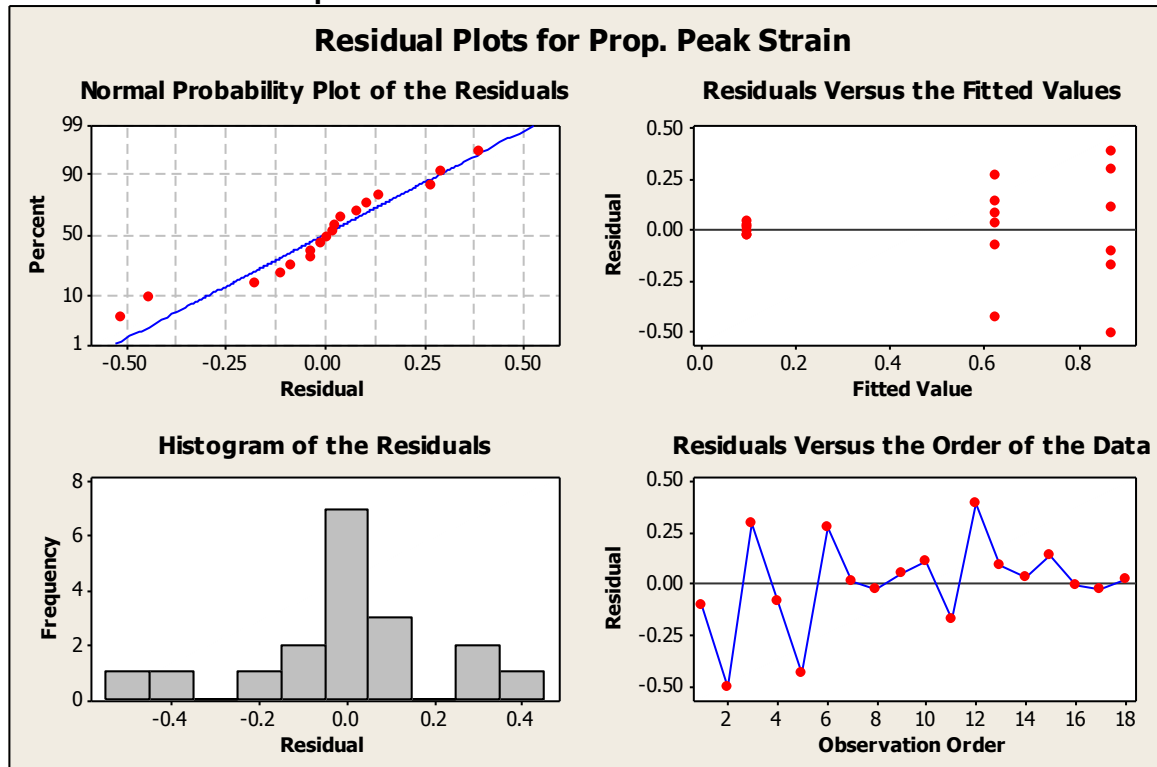
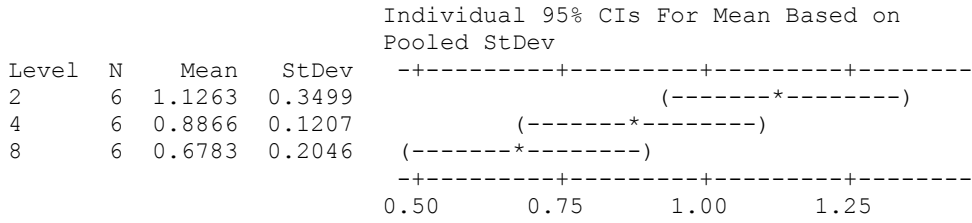


Figure 94: Residual plots for proportional peak strain, material 16

One-way ANOVA: Prop. Peak Strain versus Time for Material 17

Source	DF	SS	MS	F	P
Time	2	0.6031	0.3016	5.06	0.021
Error	15	0.8942	0.0596		
Total	17	1.4973			

S = 0.2442 R-Sq = 40.28% R-Sq(adj) = 32.32%

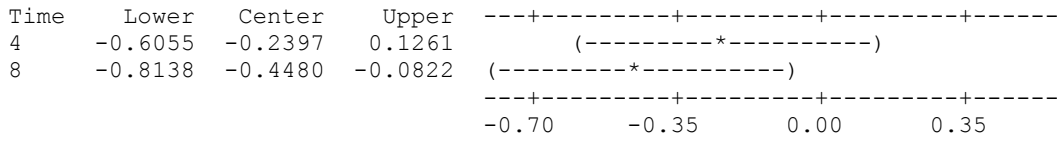


Pooled StDev = 0.2442

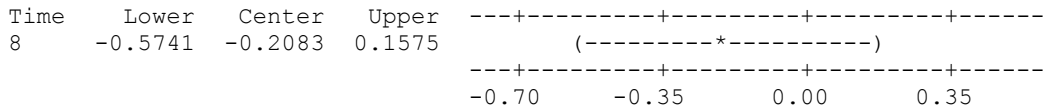
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



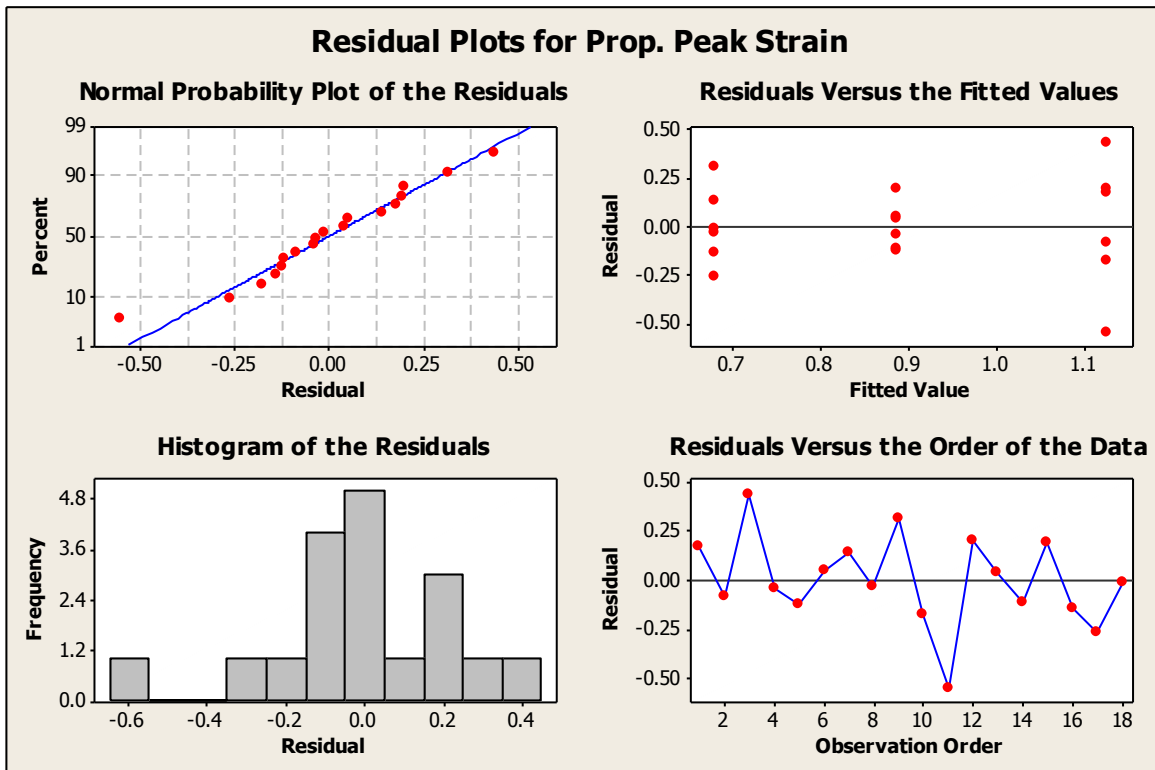


Figure 95: Residual plots for proportional peak strain, material 17

Appendix IV: Peak Stress

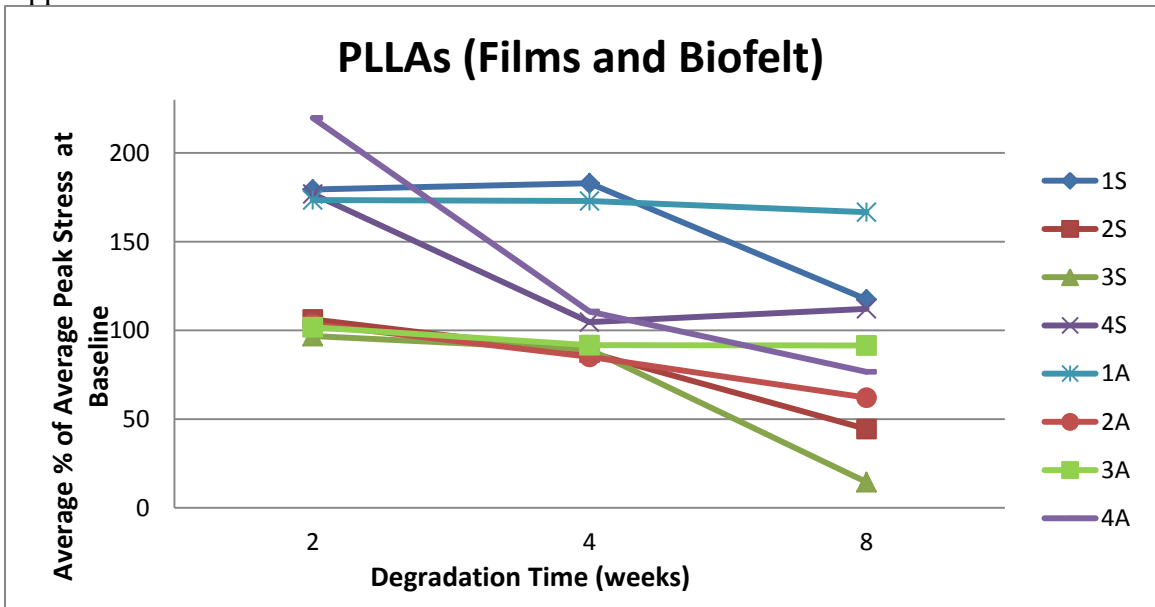


Figure 96: Average percent of baseline average peak stress in PLLA films and biofelt material (materials 1 through 4)

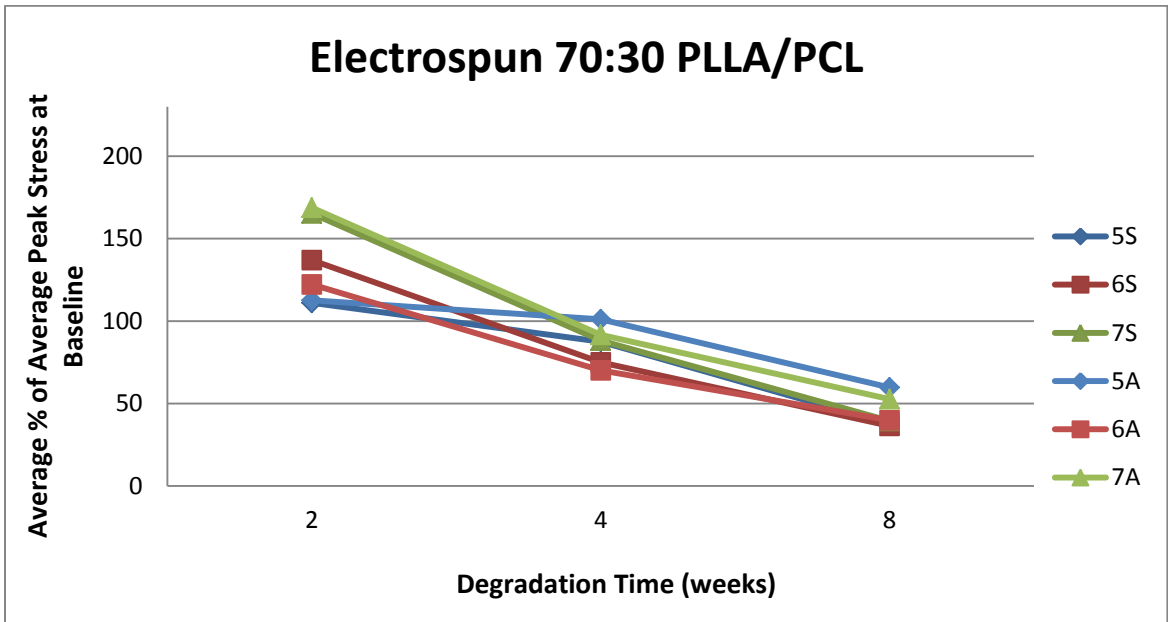


Figure 97: Average percent of baseline peak stress in 70:30 PLLA/PCL electrospun materials

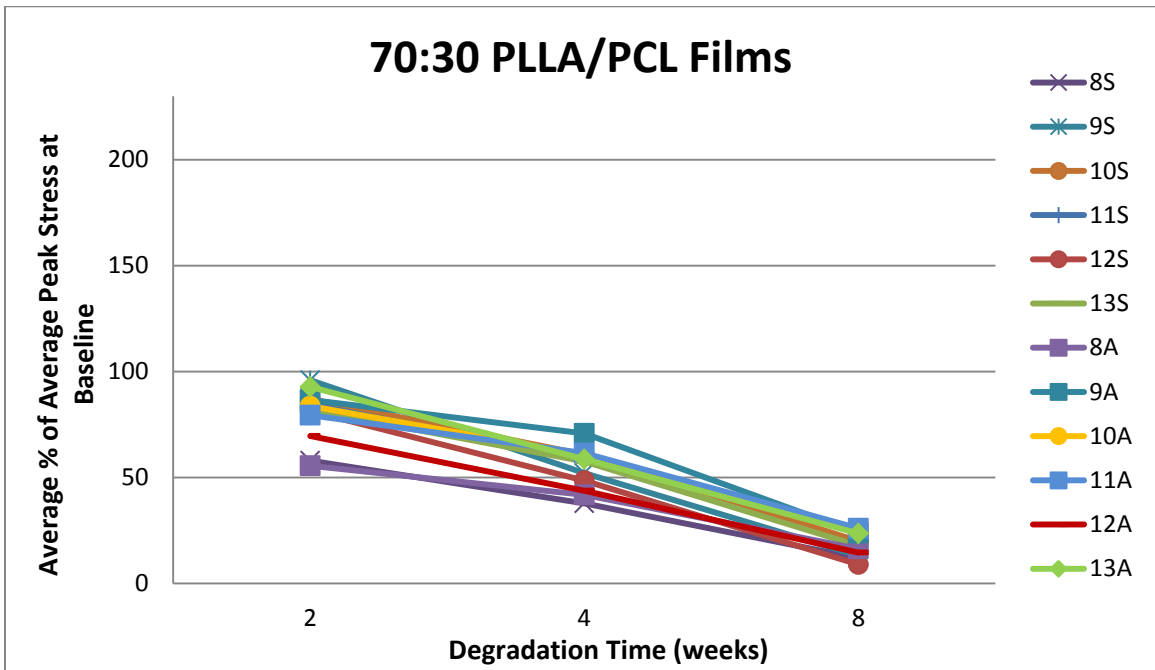


Figure 98: Average percent of average baseline peak stress in 70:30 PLLA/PCL film samples

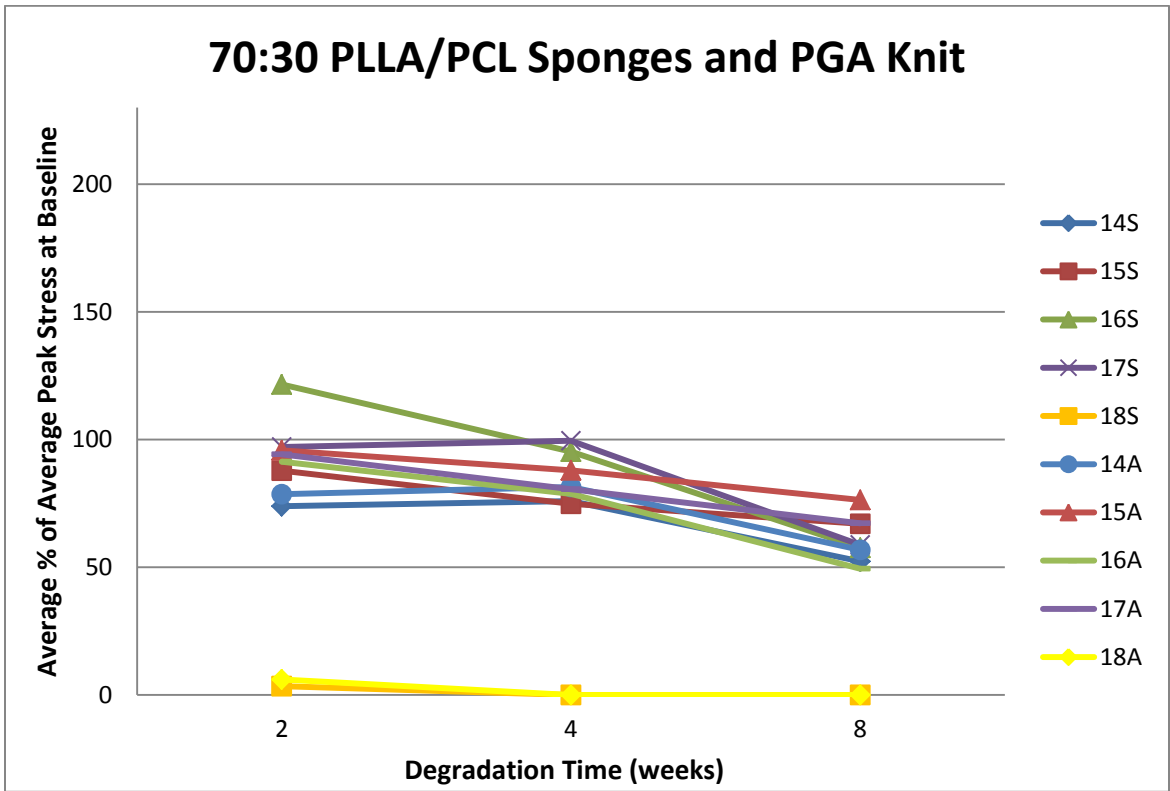


Figure 99: Average percent of baseline average peak stress in 70:30 PLLA/PCL sponges and PGA knit

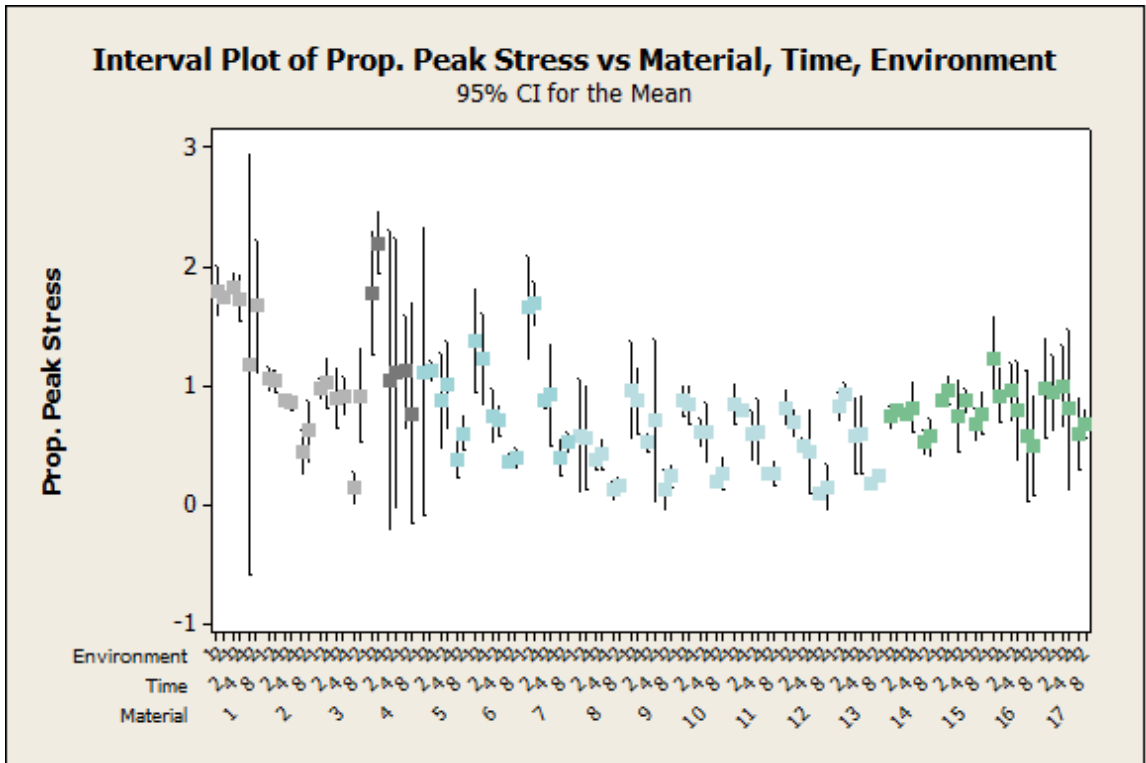


Figure 100: Interval plot of proportional peak stress by material, degradation time and degradation environment

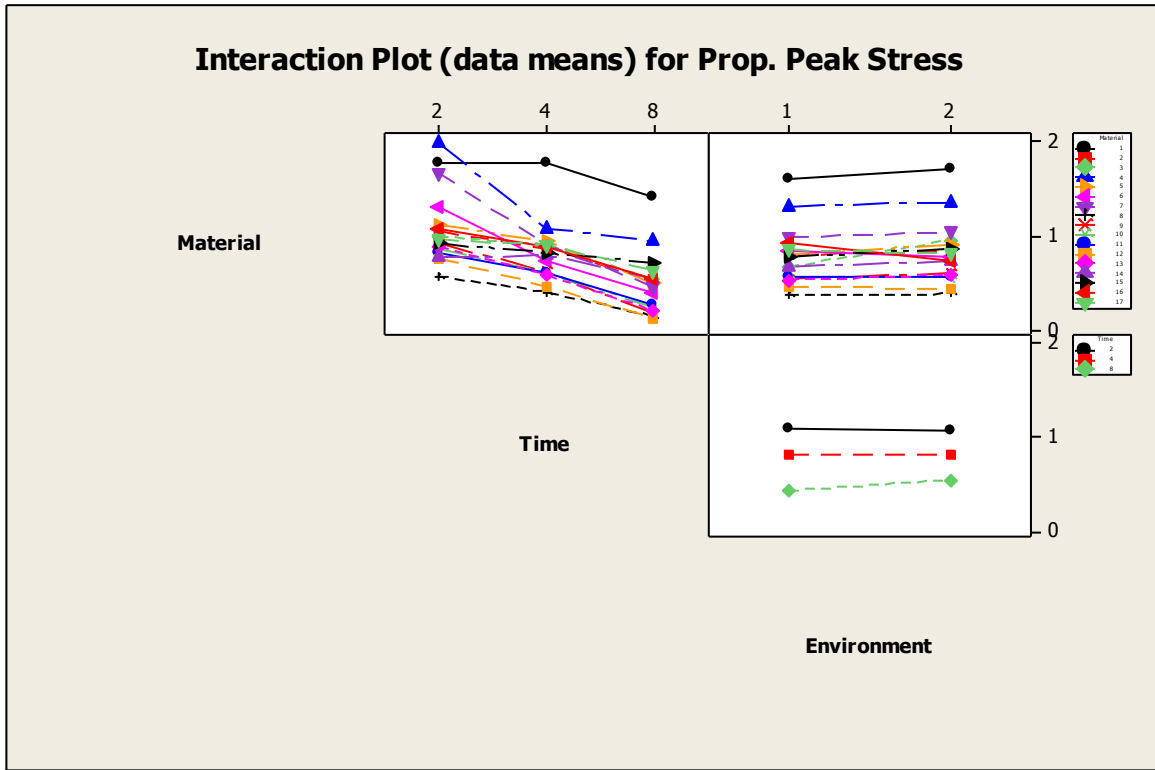


Figure 101: Interaction plot of proportional peak stress, all materials

ANOVA: Proportion of Peak Stress from Baseline versus Material, Degradation Time and Degradation Environment

Factor	Type	Levels
Material	fixed	17
Time	fixed	3
Environment	fixed	2

Factor	Values
Material	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	2, 4, 8
Environment	1, 2

Analysis of Variance for Prop. Peak Stress

Source	DF	SS	MS	F	P
Material	16	29.03511	1.81469	68.56	0.000
Time	2	17.85454	8.92727	337.28	0.000
Environment	1	0.11165	0.11165	4.22	0.041
Material*Environment	16	0.67936	0.04246	1.60	0.068
Material*Time	32	4.81372	0.15043	5.68	0.000
Time*Environment	2	0.22322	0.11161	4.22	0.016
Error	236	6.24652	0.02647		
Total	305	58.96412			

S = 0.162691 R-Sq = 89.41% R-Sq(adj) = 86.31%

Source	Variance component	Error term	Expected Mean Square for Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 102 Q[2]
3 Environment		7	(7) + 153 Q[3]
4 Material*Environment		7	(7) + 9 Q[4]
5 Material*Time		7	(7) + 6 Q[5]
6 Time*Environment		7	(7) + 51 Q[6]
7 Error	0.02647		(7)

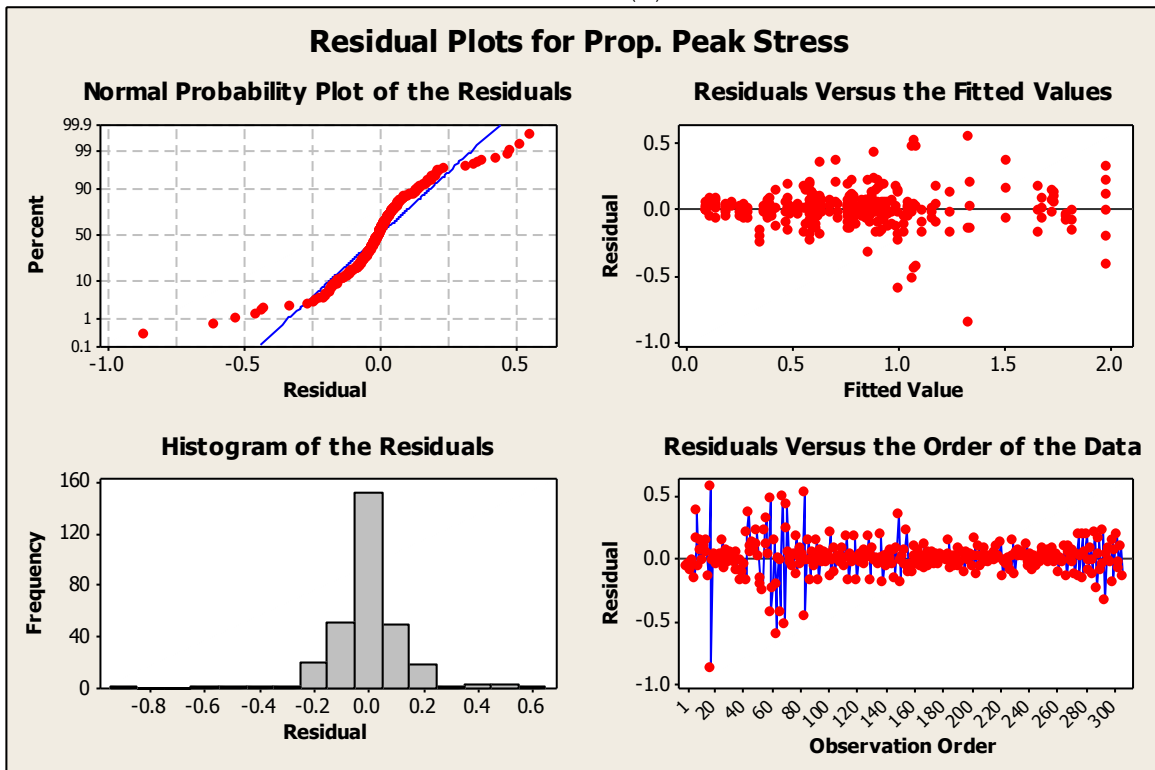


Figure 102: Residual plots of proportional peak stress, all materials

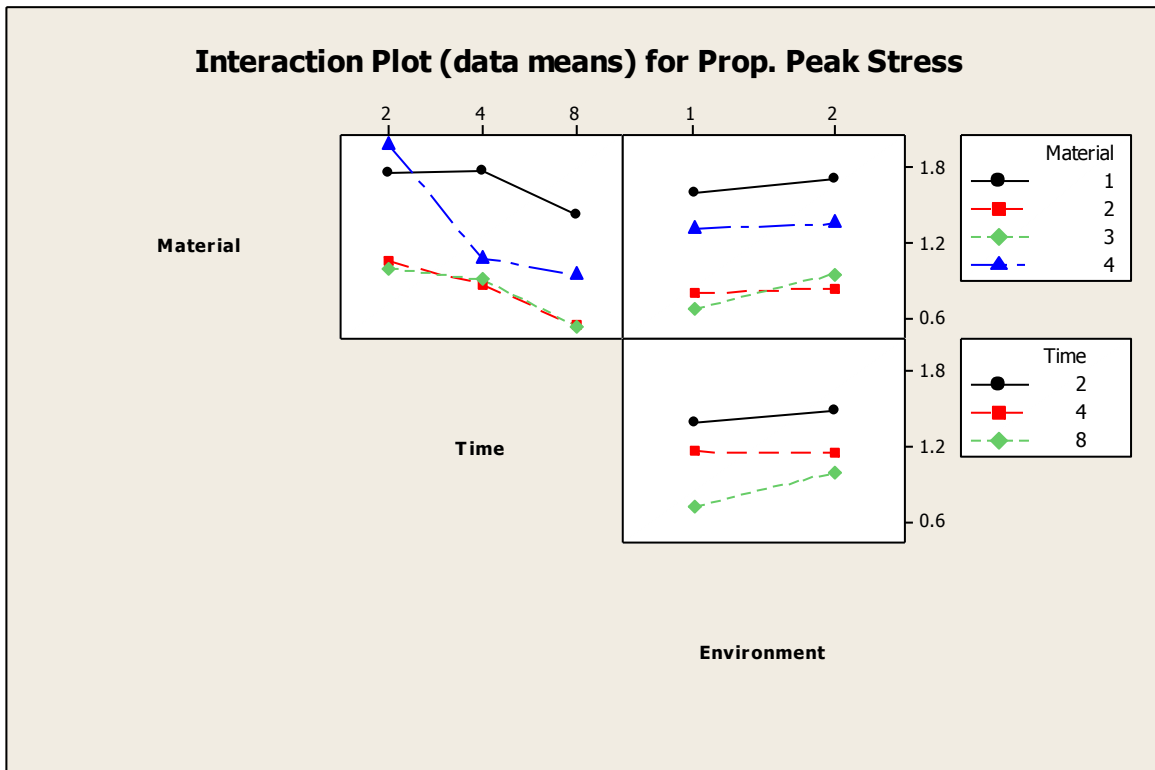


Figure 103: Interaction plot of proportional peak stress, materials 1-4

ANOVA: Prop. Peak Stress versus Material, Time, Environment

Factor	Type	Levels	Values
Material	fixed	4	1, 2, 3, 4
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Prop. Peak Stress

Source	DF	SS	MS	F	P
Material	3	9.30883	3.10294	44.12	0.000
Time	2	4.18560	2.09280	29.75	0.000
Environment	1	0.25714	0.25714	3.66	0.061
Material*Environment	3	0.17022	0.05674	0.81	0.496
Material*Time	6	1.69213	0.28202	4.01	0.002
Time*Environment	2	0.24088	0.12044	1.71	0.190
Error	54	3.79806	0.07033		
Total	71	19.65286			

S = 0.265206 R-Sq = 80.67% R-Sq(adj) = 74.59%

Expected Mean
Square for
Each Term
(using

Source	Variance component	Error term	restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 24 Q[2]
3 Environment		7	(7) + 36 Q[3]
4 Material*Environment		7	(7) + 9 Q[4]
5 Material*Time		7	(7) + 6 Q[5]
6 Time*Environment		7	(7) + 12 Q[6]
7 Error	0.07033		(7)

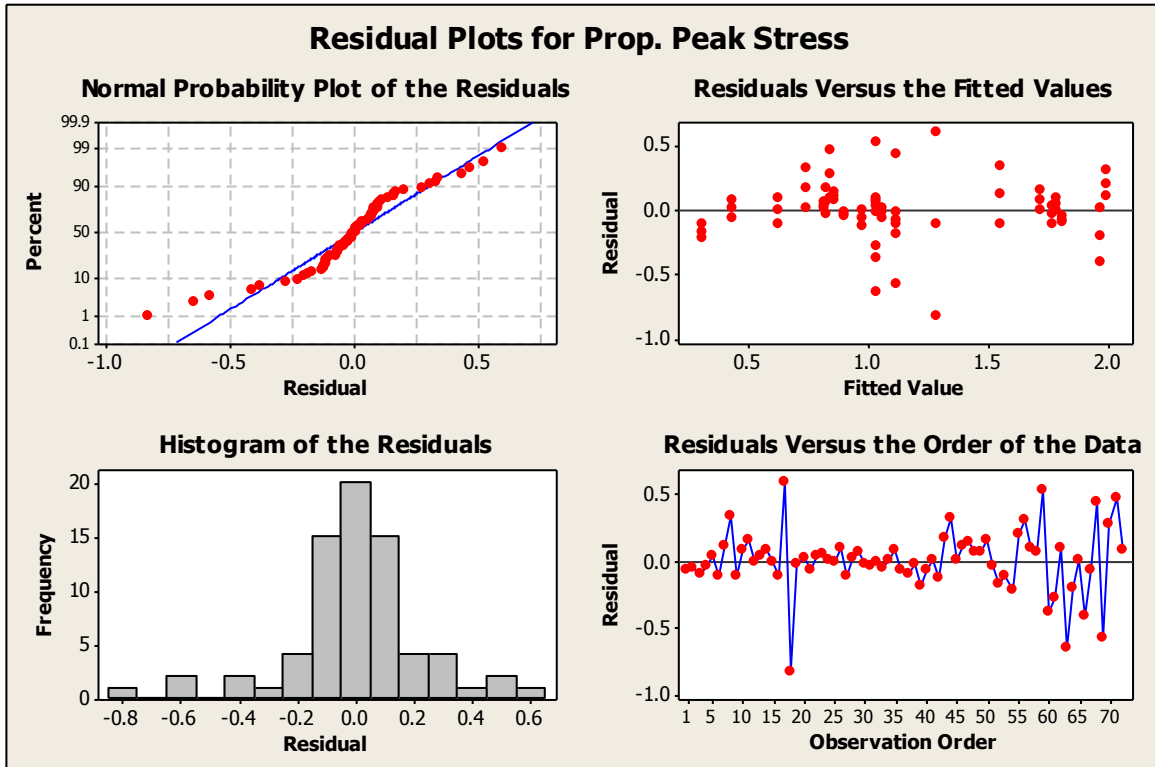


Figure 104: Residual plots of proportional peak stress, materials 1-4

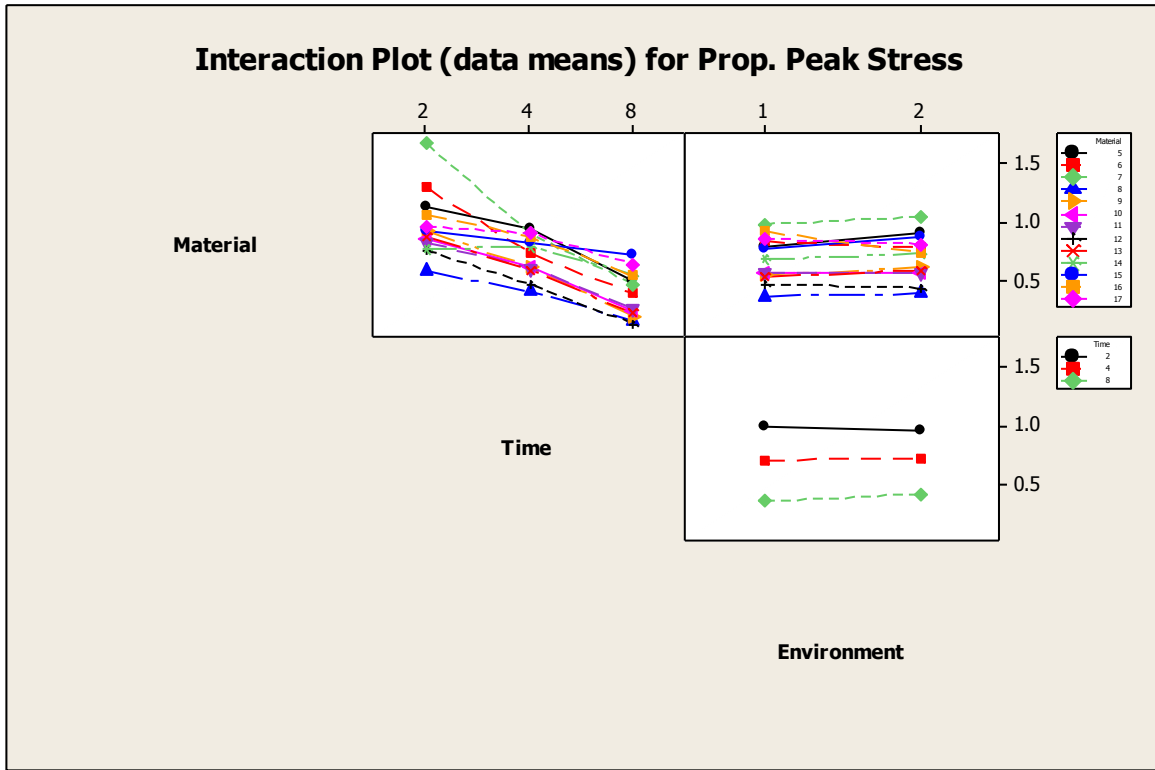


Figure 105: Interaction plot of proportional peak stress, materials 5-17

ANOVA: Proportional Peak Stress from Baseline versus Material, Degradation Time and Degradation Environment (materials 5-17)

Factor	Type	Levels	Values
Material	fixed	13	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17
Time	fixed	3	2, 4, 8
Environment	fixed	2	1, 2

Analysis of Variance for Prop. Peak Stress

Source	DF	SS	MS	F	P
Material	12	7.58489	0.63207	48.99	0.000
Time	2	13.68160	6.84080	530.20	0.000
Environment	1	0.01017	0.01017	0.79	0.376
Material*Environment	12	0.35348	0.02946	2.28	0.010
Material*Time	24	3.10893	0.12954	10.04	0.000
Time*Environment	2	0.10839	0.05420	4.20	0.016
Error	180	2.32240	0.01290		
Total	233	27.16986			

S = 0.113588 R-Sq = 91.45% R-Sq(adj) = 88.94%

Expected Mean

Source	Variance component	Error term	Square for Each Term (using restricted model)
1 Material		7	(7) + 18 Q[1]
2 Time		7	(7) + 78 Q[2]
3 Environment		7	(7) + 117 Q[3]
4 Material*Environment		7	(7) + 9 Q[4]
5 Material*Time		7	(7) + 6 Q[5]
6 Time*Environment		7	(7) + 39 Q[6]
7 Error	0.01290		(7)

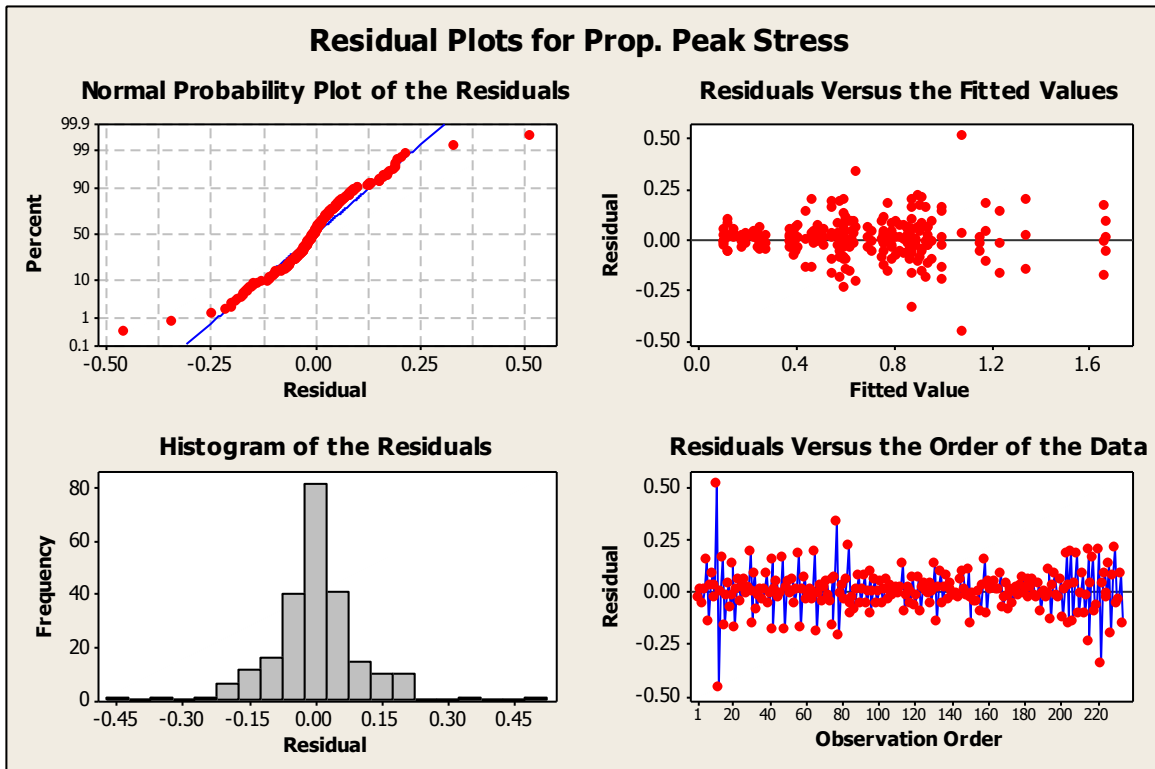


Figure 106: Residual plots for proportional peak stress, materials 5-17

Minitab Project Report

One-way ANOVA: Prop. Peak Stress versus Time for Material 1

Source	DF	SS	MS	F	P
Time	2	0.495	0.248	2.43	0.121
Error	15	1.526	0.102		
Total	17	2.021			

S = 0.3190 R-Sq = 24.50% R-Sq(adj) = 14.44%

Individual 95% CIs For Mean Based on
Pooled StDev

Level	N	Mean	StDev
2	6	1.7645	0.0635
4	6	1.7796	0.0784
8	6	1.4204	0.5432

-----+-----+-----+-----+-----
 (-----*-----)
 (-----*-----)
 (-----*-----)
 -----+-----+-----+-----+-----
 1.25 1.50 1.75 2.00

Pooled StDev = 0.3190

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.4627	0.0152	0.4931
8	-0.8219	-0.3440	0.1338

-----+-----+-----+-----+-----
 (-----*-----)
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.50 0.00 0.50 1.00

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.8371	-0.3592	0.1186

-----+-----+-----+-----+-----
 (-----*-----)
 -----+-----+-----+-----+-----
 -0.50 0.00 0.50 1.00

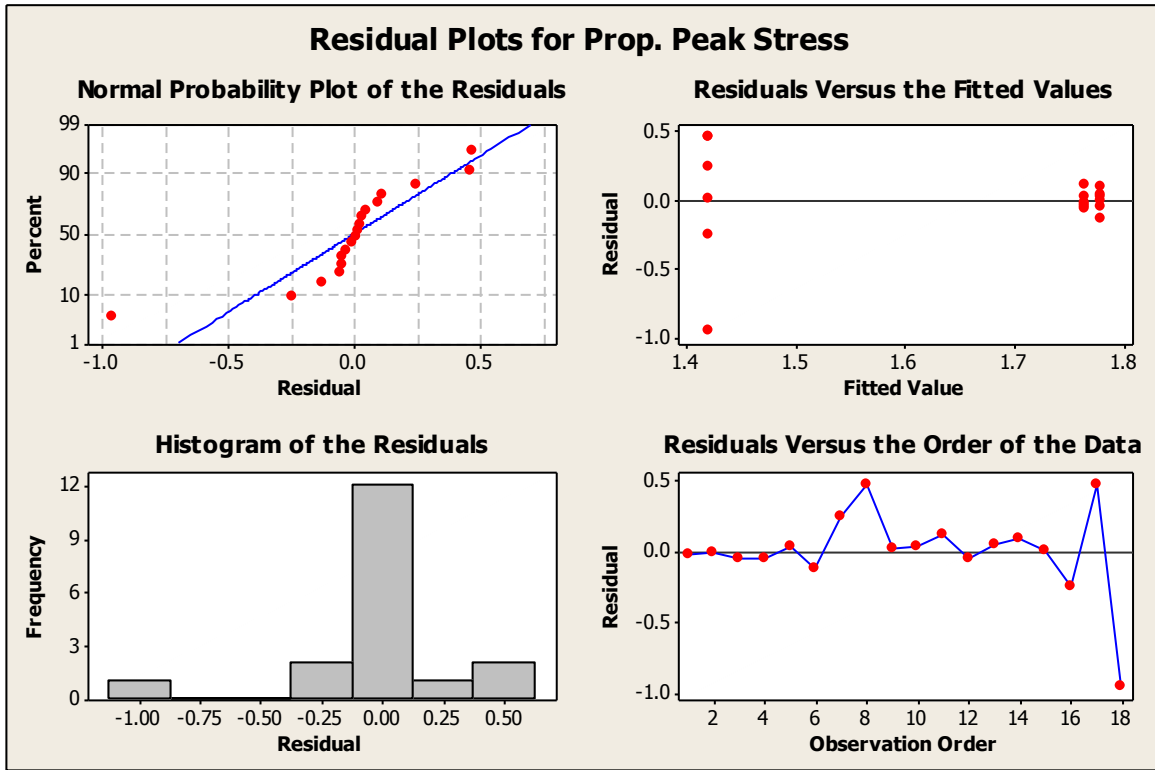


Figure 107: Residual plots for proportional peak stress, material 1

One-way ANOVA: Prop. Peak Stress versus Time for Material 2

Source	DF	SS	MS	F	P
Time	2	0.82103	0.41051	68.01	0.000
Error	15	0.09054	0.00604		
Total	17	0.91156			

S = 0.07769 R-Sq = 90.07% R-Sq(adj) = 88.74%

Level	N	Mean	StDev
2	6	1.0491	0.0385
4	6	0.8632	0.0239
8	6	0.5327	0.1267

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 0.0777

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.30230	-0.18590	-0.06950
8	-0.63283	-0.51643	-0.40003

-----+-----+-----+-----+-----
 (---*---)
 (---*---)
 -----+-----+-----+-----+-----

-0.60 -0.30 0.00 0.30

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.44694	-0.33053	-0.21413

-----+-----+-----+-----+-----
 (---*---)
 -----+-----+-----+-----+-----

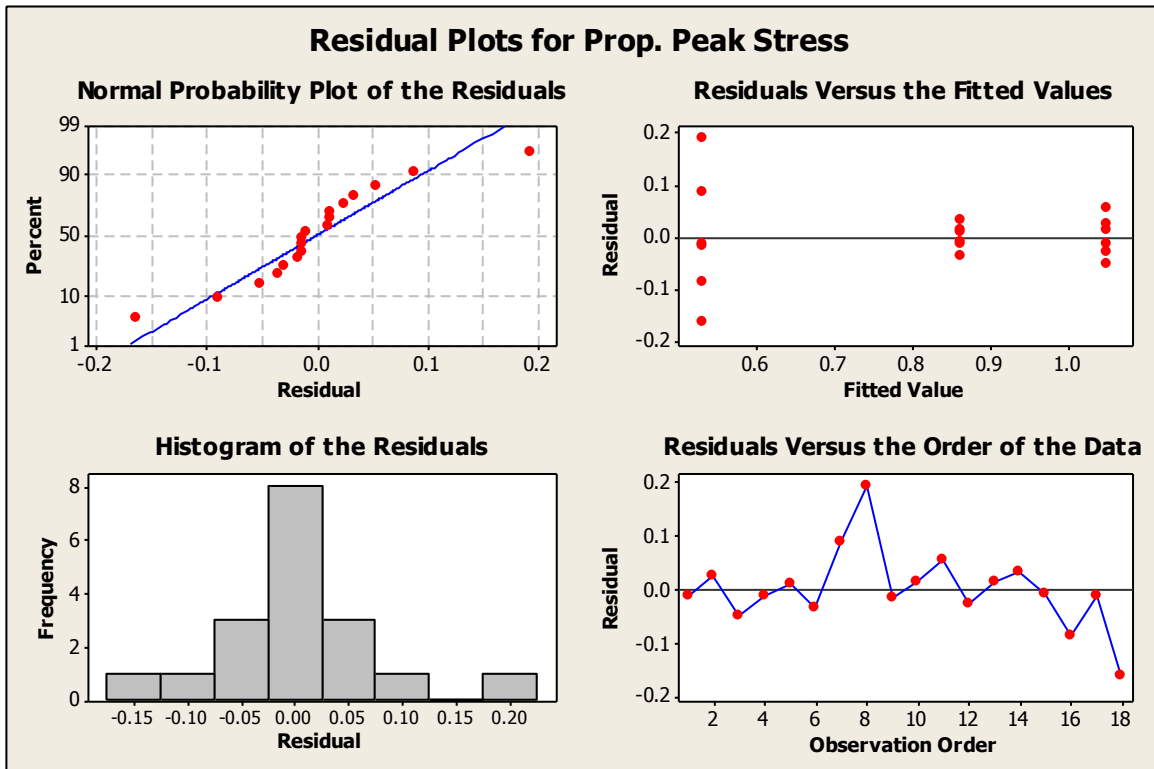


Figure 108: Residual plots for proportional peak stress, material 2

One-way ANOVA: Prop. Peak Stress versus Time for Material 3

Source	DF	SS	MS	F	P
Time	2	0.7241	0.3620	5.46	0.017
Error	15	0.9943	0.0663		
Total	17	1.7184			

S = 0.2575 R-Sq = 42.14% R-Sq(adj) = 34.42%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev

-----+-----+-----+-----+-----

2	6	0.9929	0.0620	(-----*-----)
4	6	0.9046	0.0763	(-----*-----)
8	6	0.5302	0.4350	(-----*-----)

-----+-----+-----+-----+-----+-----+-----
 0.50 0.75 1.00 1.25

Pooled StDev = 0.2575

Tukey 95% Simultaneous Confidence Intervals
 All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----+-----+-----
4	-0.4740	-0.0883	0.2975	(-----*-----)
8	-0.8484	-0.4627	-0.0769	(-----*-----)

-----+-----+-----+-----+-----+-----+-----
 -0.50 0.00 0.50 1.00

Time = 4 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----+-----+-----
8	-0.7601	-0.3744	0.0114	(-----*-----)

-----+-----+-----+-----+-----+-----+-----
 -0.50 0.00 0.50 1.00

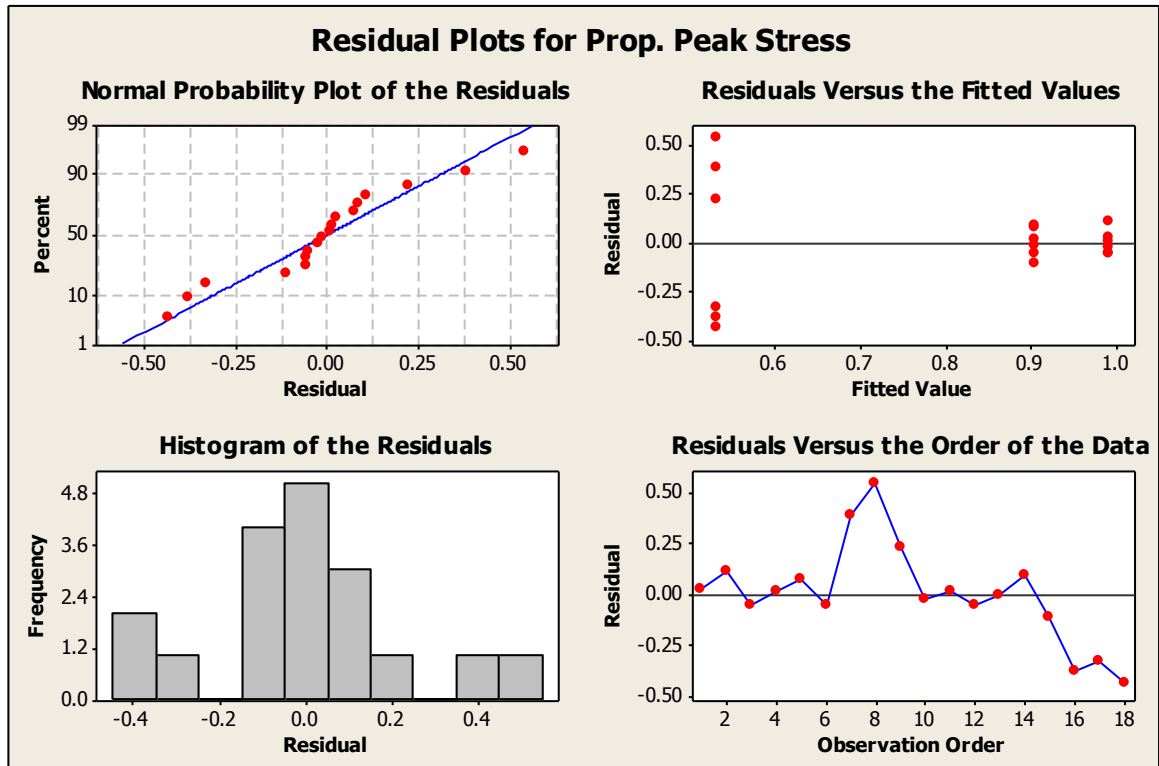
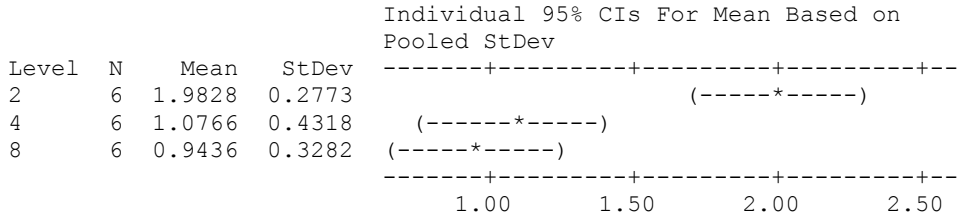


Figure 109: Residual plots for proportional peak stress, material 3

One-way ANOVA: Prop. Peak Stress versus Time for Material 4

Source	DF	SS	MS	F	P
Time	2	3.837	1.919	15.51	0.000
Error	15	1.855	0.124		
Total	17	5.693			

S = 0.3517 R-Sq = 67.41% R-Sq(adj) = 63.06%

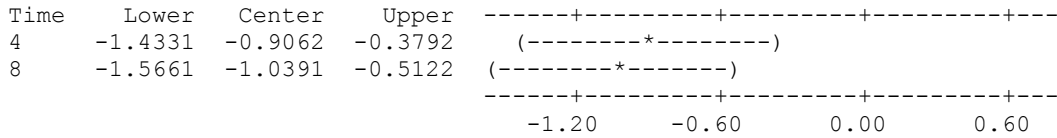


Pooled StDev = 0.3517

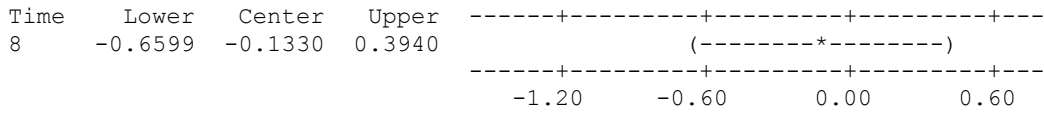
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



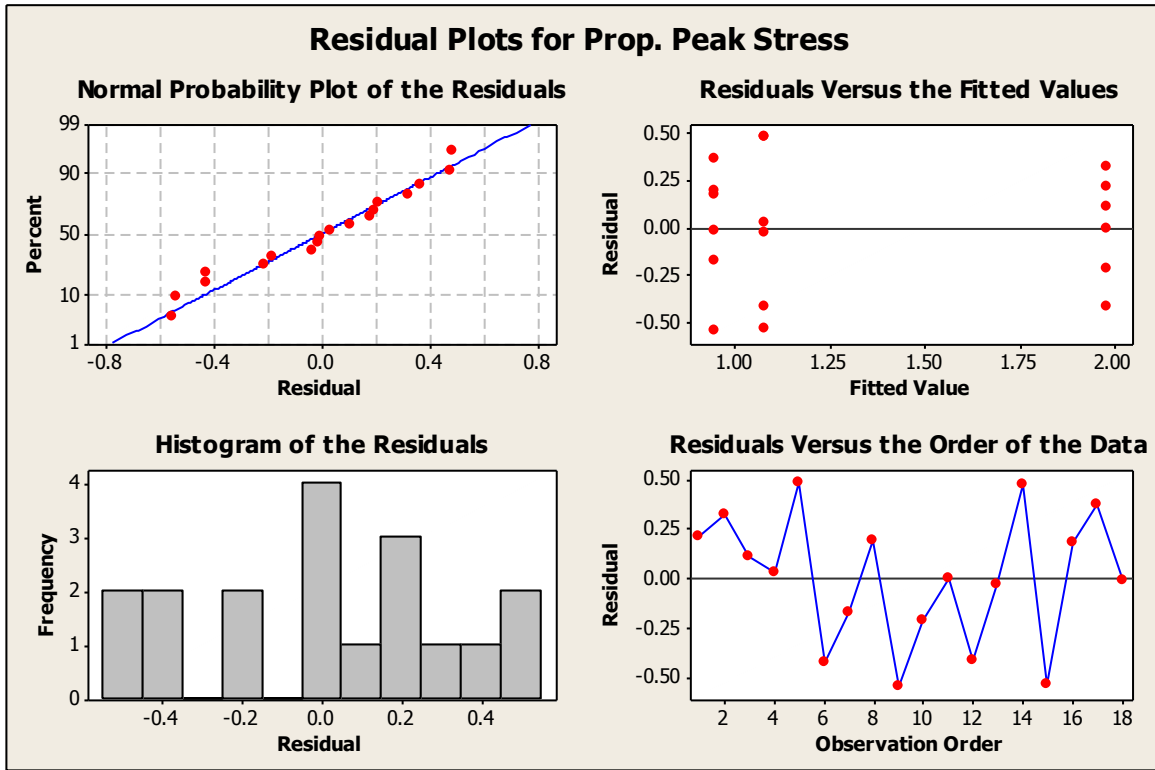
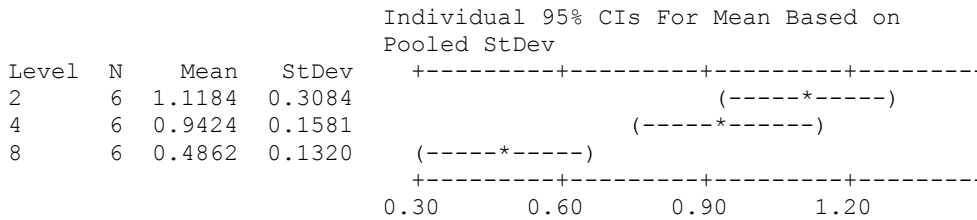


Figure 110: Residual plots for proportional peak stress, material 4

One-way ANOVA: Prop. Peak Stress versus Time for Material 5

Source	DF	SS	MS	F	P
Time	2	1.2775	0.6387	13.93	0.000
Error	15	0.6877	0.0458		
Total	17	1.9652			

S = 0.2141 R-Sq = 65.01% R-Sq(adj) = 60.34%



Pooled StDev = 0.2141

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.4969	-0.1760	0.1448
8	-0.9530	-0.6322	-0.3114

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.7770	-0.4562	-0.1353

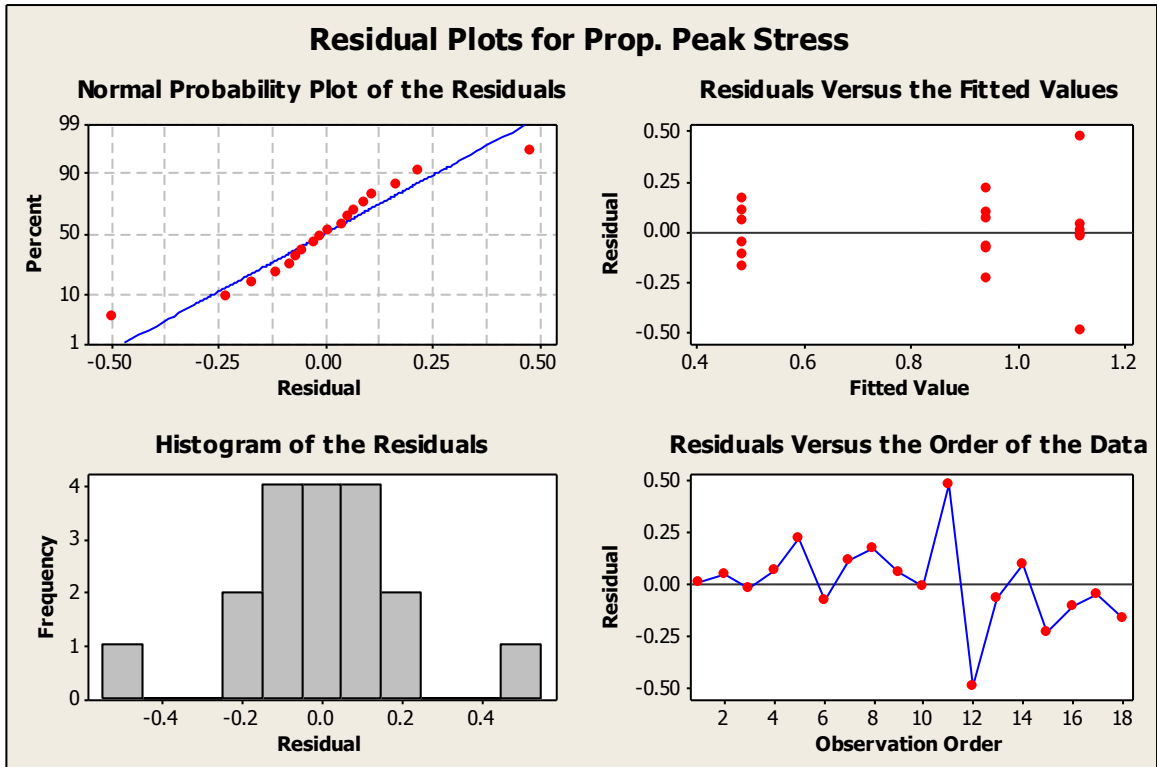


Figure 111: Residual plots for proportional peak stress, material 5

One-way ANOVA: Prop. Peak Stress versus Time for Material 6

Source	DF	SS	MS	F	P
Time	2	2.5599	1.2799	112.72	0.000
Error	15	0.1703	0.0114		
Total	17	2.7302			

S = 0.1066 R-Sq = 93.76% R-Sq(adj) = 92.93%

Level	N	Mean	StDev
Individual 95% CIs For Mean Based on Pooled StDev			

2	6	1.2960	0.1680	(---*--)
4	6	0.7254	0.0696	(---*--)
8	6	0.3816	0.0313	(---*--)

Pooled StDev = 0.1066

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----
4	-0.7302	-0.5706	-0.4109	(---*---)
8	-1.0741	-0.9144	-0.7548	(---*---)

Time = 4 subtracted from:

Time	Lower	Center	Upper	-----+-----+-----+-----+-----
8	-0.5035	-0.3438	-0.1842	(---*---)

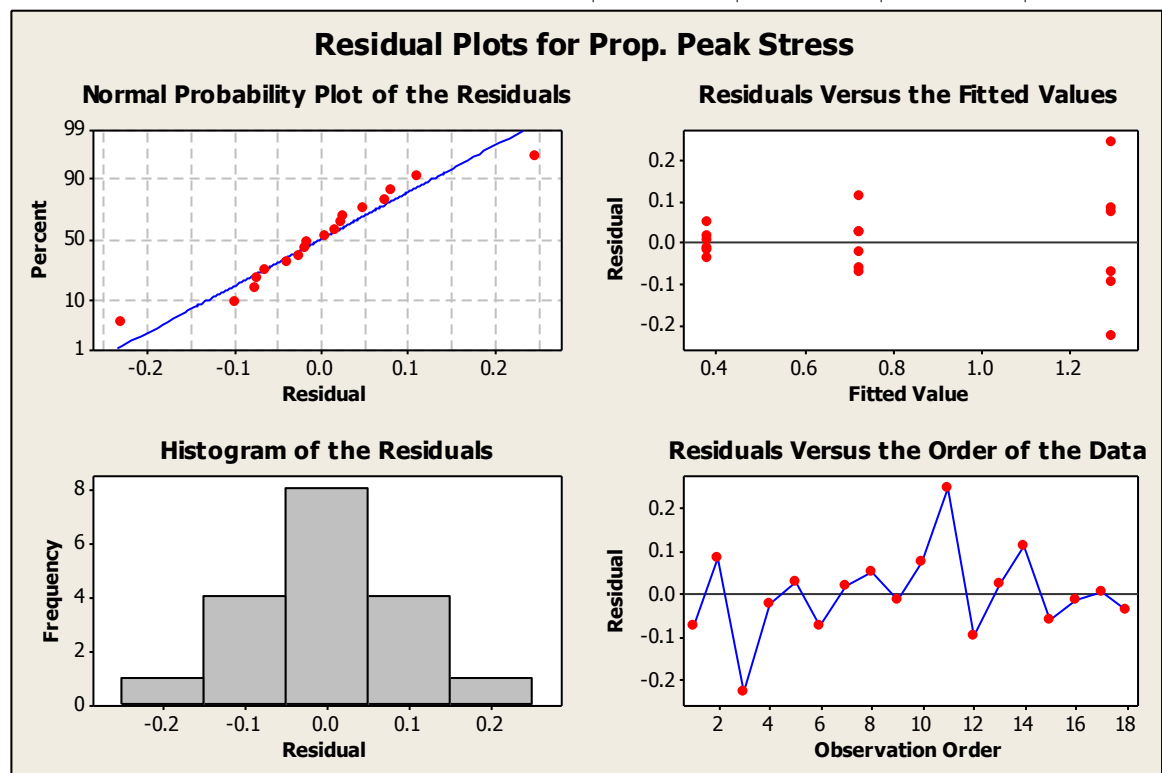
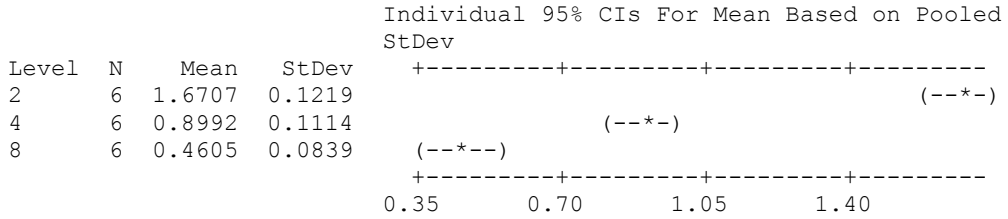


Figure 112: Residual plots for proportional peak stress, material 6

One-way ANOVA: Prop. Peak Stress versus Time for Material 7

Source	DF	SS	MS	F	P
Time	2	4.5041	2.2521	196.85	0.000
Error	15	0.1716	0.0114		
Total	17	4.6757			

S = 0.1070 R-Sq = 96.33% R-Sq(adj) = 95.84%

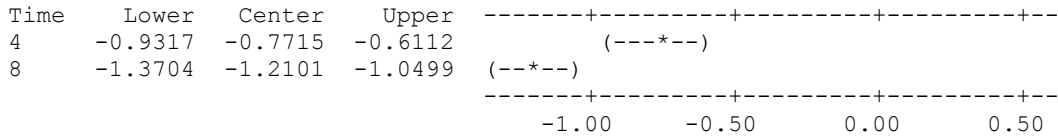


Pooled StDev = 0.1070

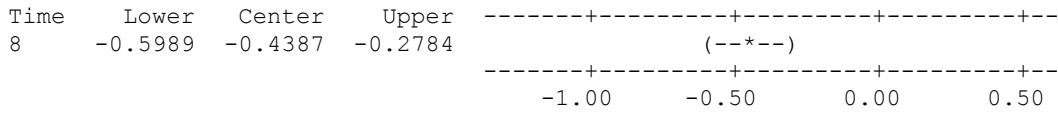
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



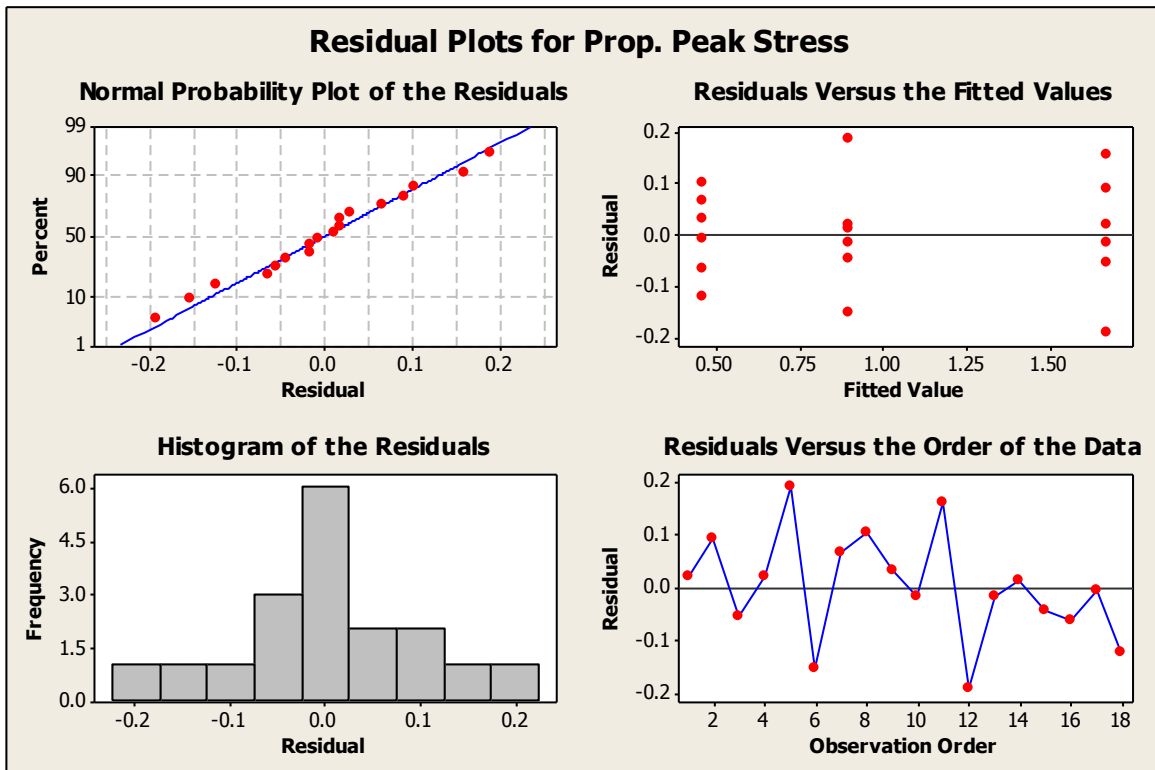
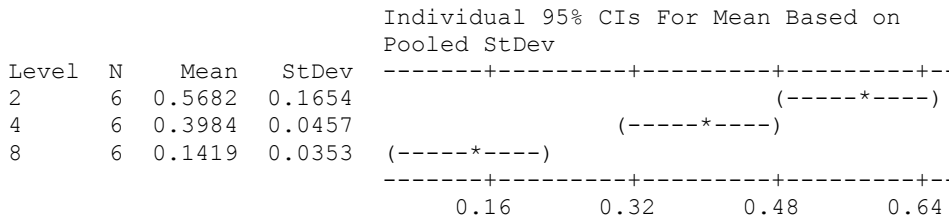


Figure 113: Residual plots for proportional peak stress, material 7

One-way ANOVA: Prop. Peak Stress versus Time for Material 8

Source	DF	SS	MS	F	P
Time	2	0.5525	0.2763	27.00	0.000
Error	15	0.1535	0.0102		
Total	17	0.7061			

S = 0.1012 R-Sq = 78.26% R-Sq(adj) = 75.36%



Pooled StDev = 0.1012

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.3214	-0.1698	-0.0182
8	-0.5778	-0.4262	-0.2747

(-----*-----)
(-----*-----)

-0.50 -0.25 0.00 0.25

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.4080	-0.2564	-0.1049

(-----*-----)

-0.50 -0.25 0.00 0.25

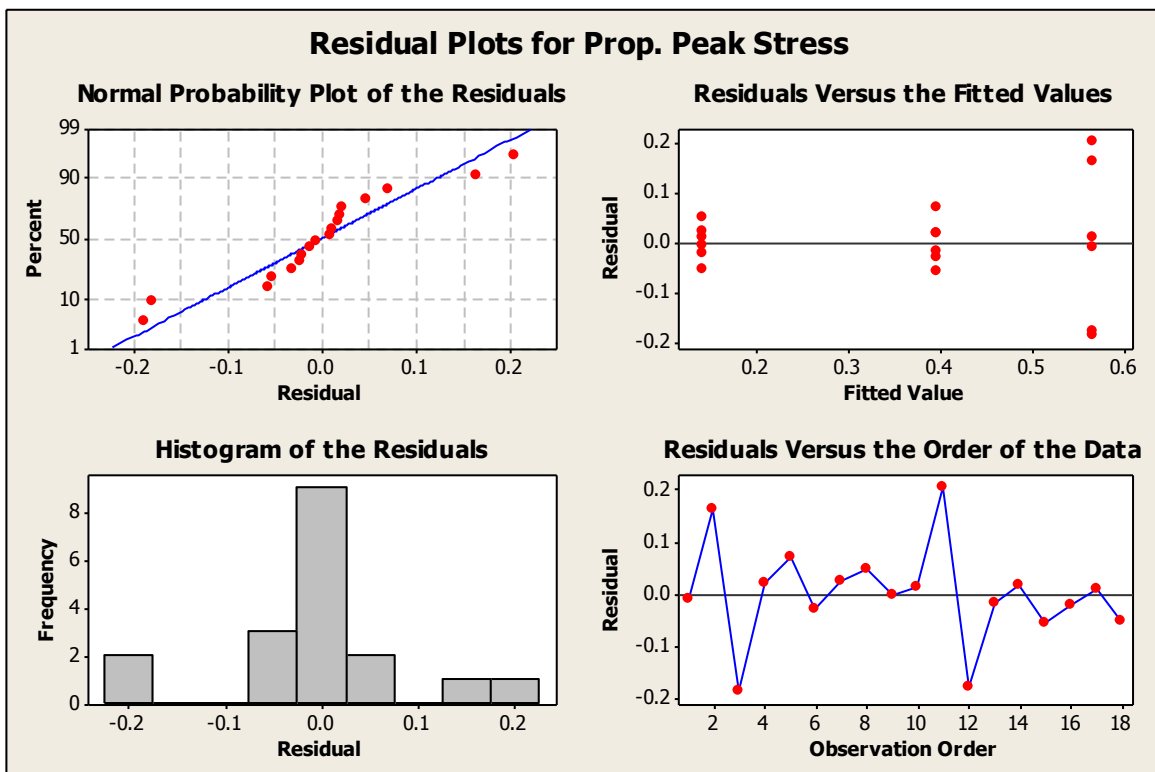


Figure 114: Residual plots for proportional peak stress, material 8

One-way ANOVA: Prop. Peak Stress versus Time for Material 9

Source	DF	SS	MS	F	P
Time	2	1.6085	0.8043	37.26	0.000
Error	15	0.3238	0.0216		
Total	17	1.9323			

S = 0.1469 R-Sq = 83.24% R-Sq(adj) = 81.01%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
2	6	0.9133	0.1349
4	6	0.6144	0.2021
8	6	0.1850	0.0755

-----+-----+-----+-----+

(-----*-----)

(-----*-----)

(-----*-----)

-----+-----+-----+-----+

0.25 0.50 0.75 1.00

Pooled StDev = 0.1469

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.5190	-0.2989	-0.0788
8	-0.9485	-0.7283	-0.5082

-----+-----+-----+-----+

(-----*-----)

(-----*-----)

-----+-----+-----+-----+

-0.80 -0.40 -0.00 0.40

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.6496	-0.4295	-0.2093

-----+-----+-----+-----+

(-----*-----)

-----+-----+-----+-----+

-0.80 -0.40 -0.00 0.40

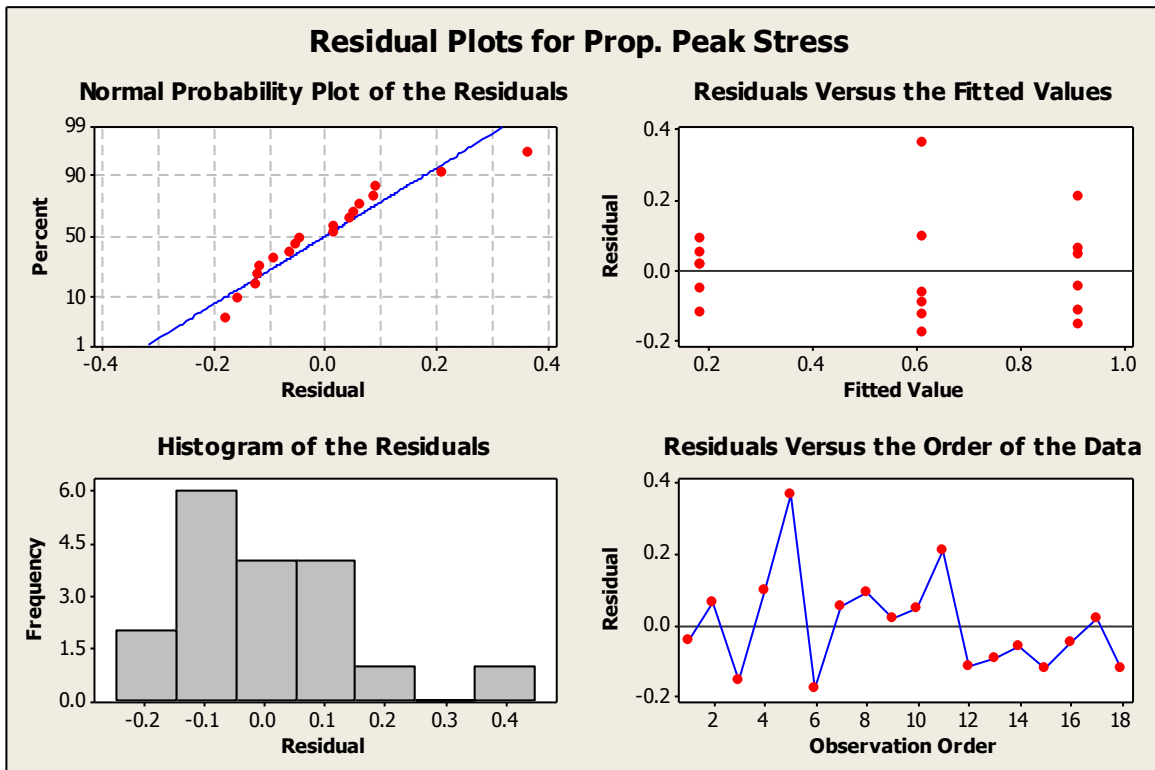


Figure 115: Residual plots for proportional peak stress, material 9

One-way ANOVA: Prop. Peak Stress versus Time for Material 10

Source	DF	SS	MS	F	P
Time	2	1.19357	0.59678	177.84	0.000
Error	15	0.05033	0.00336		
Total	17	1.24390			

S = 0.05793 R-Sq = 95.95% R-Sq(adj) = 95.41%

Level	N	Mean	StDev
2	6	0.85407	0.05463
4	6	0.60734	0.06882
8	6	0.22798	0.04844

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 0.05793

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.33352	-0.24673	-0.15993
8	-0.71288	-0.62609	-0.53930

-----+-----+-----+-----+-----
 (---*---) (---*---)
 -----+-----+-----+-----+-----
 -0.60 -0.30 0.00 0.30

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.46616	-0.37937	-0.29257

-----+-----+-----+-----+-----
 (---*---)
 -----+-----+-----+-----+-----
 -0.60 -0.30 0.00 0.30

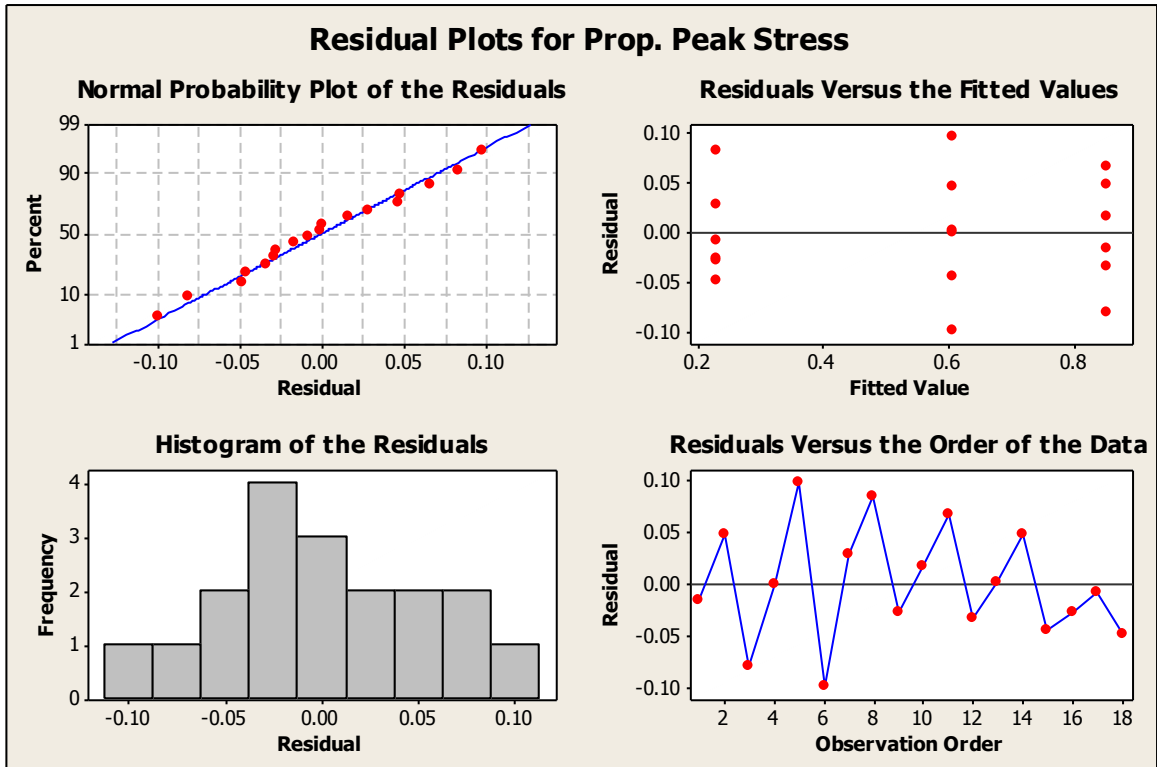


Figure 116: Residual plots for proportional peak stress, material 10

One-way ANOVA: Prop. Peak Stress versus Time for Material 11

Source	DF	SS	MS	F	P
Time	2	0.97059	0.48529	127.34	0.000
Error	15	0.05717	0.00381		
Total	17	1.02775			

S = 0.06173 R-Sq = 94.44% R-Sq(adj) = 93.70%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	+-----+-----+-----+-----+-----

2	6	0.82045	0.05209	(---*)
4	6	0.60027	0.08948	(---*)
8	6	0.25617	0.02672	(---*)

Pooled StDev = 0.06173

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper	(---*)
4	-0.31268	-0.22018	-0.12769	(---*)
8	-0.65677	-0.56428	-0.47179	(---*)

Time = 4 subtracted from:

Time	Lower	Center	Upper	(---*)
8	-0.43659	-0.34410	-0.25160	(---*)

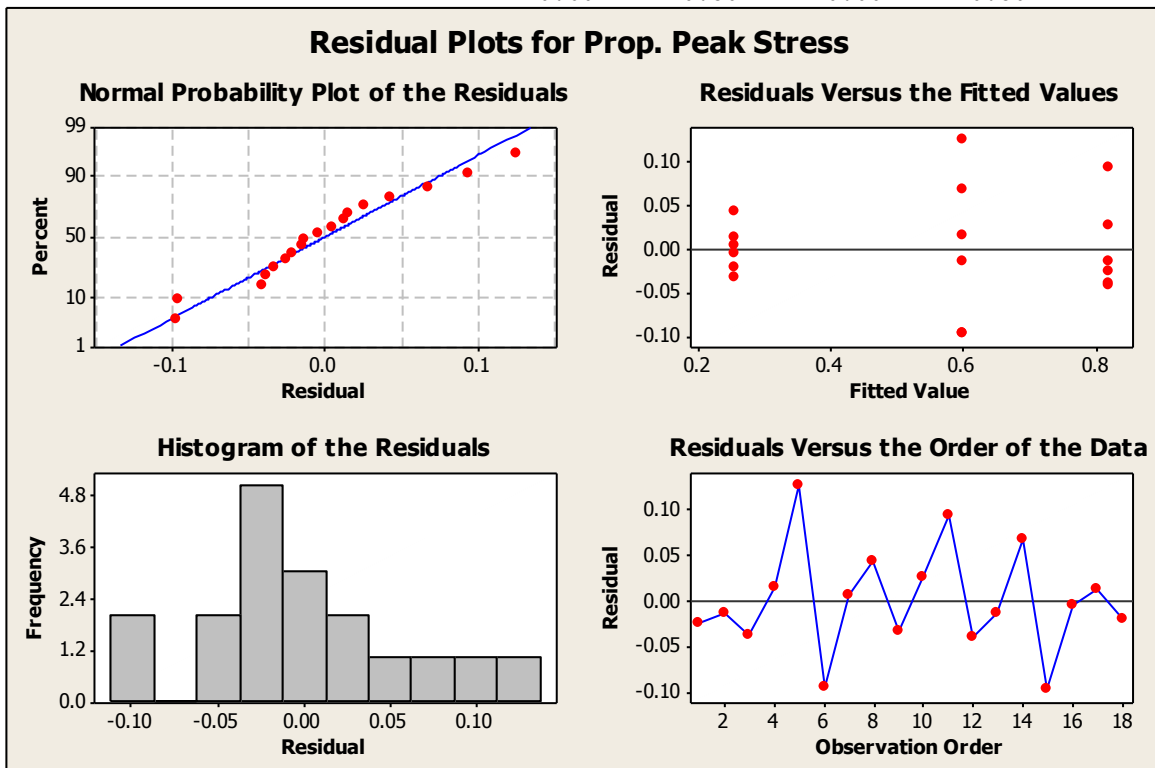
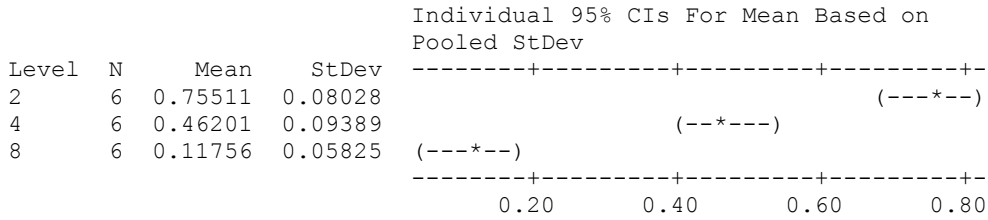


Figure 117: Residual plots for proportional peak stress, material 11

One-way ANOVA: Prop. Peak Stress versus Time for Material 12

Source	DF	SS	MS	F	P
Time	2	1.22205	0.61103	98.27	0.000
Error	15	0.09327	0.00622		
Total	17	1.31532			

S = 0.07885 R-Sq = 92.91% R-Sq(adj) = 91.96%

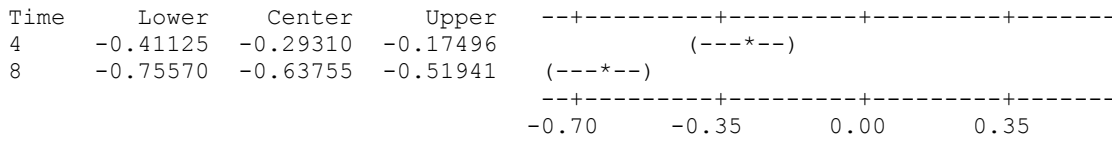


Pooled StDev = 0.07885

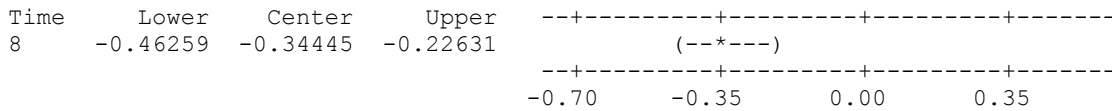
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



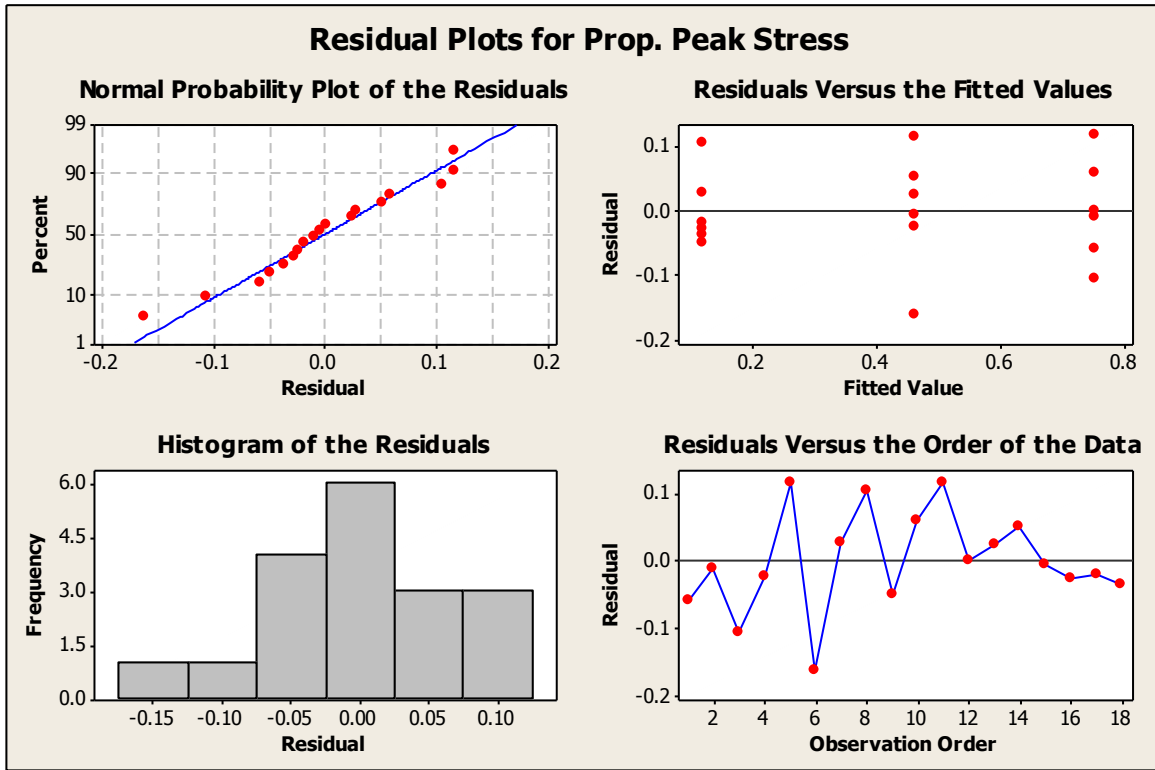


Figure 118: Residual plots for proportional peak stress, material 12

One-way ANOVA: Prop. Peak Stress versus Time for Material 13

Source	DF	SS	MS	F	P
Time	2	1.34169	0.67085	100.72	0.000
Error	15	0.09991	0.00666		
Total	17	1.44160			

S = 0.08161 R-Sq = 93.07% R-Sq(adj) = 92.15%

Level	N	Mean	StDev
2	6	0.87432	0.07114
4	6	0.58145	0.11635
8	6	0.20722	0.03720

Individual 95% CIs For Mean Based on Pooled StDev

Pooled StDev = 0.08161

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.41515	-0.29287	-0.17060
8	-0.78938	-0.66710	-0.54482

-----+-----+-----+-----+-----
 (---*---)
 (---*---)
 -----+-----+-----+-----+-----
 -0.70 -0.35 0.00 0.35

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.49651	-0.37423	-0.25195

-----+-----+-----+-----+-----
 (---*---)
 -----+-----+-----+-----+-----
 -0.70 -0.35 0.00 0.35

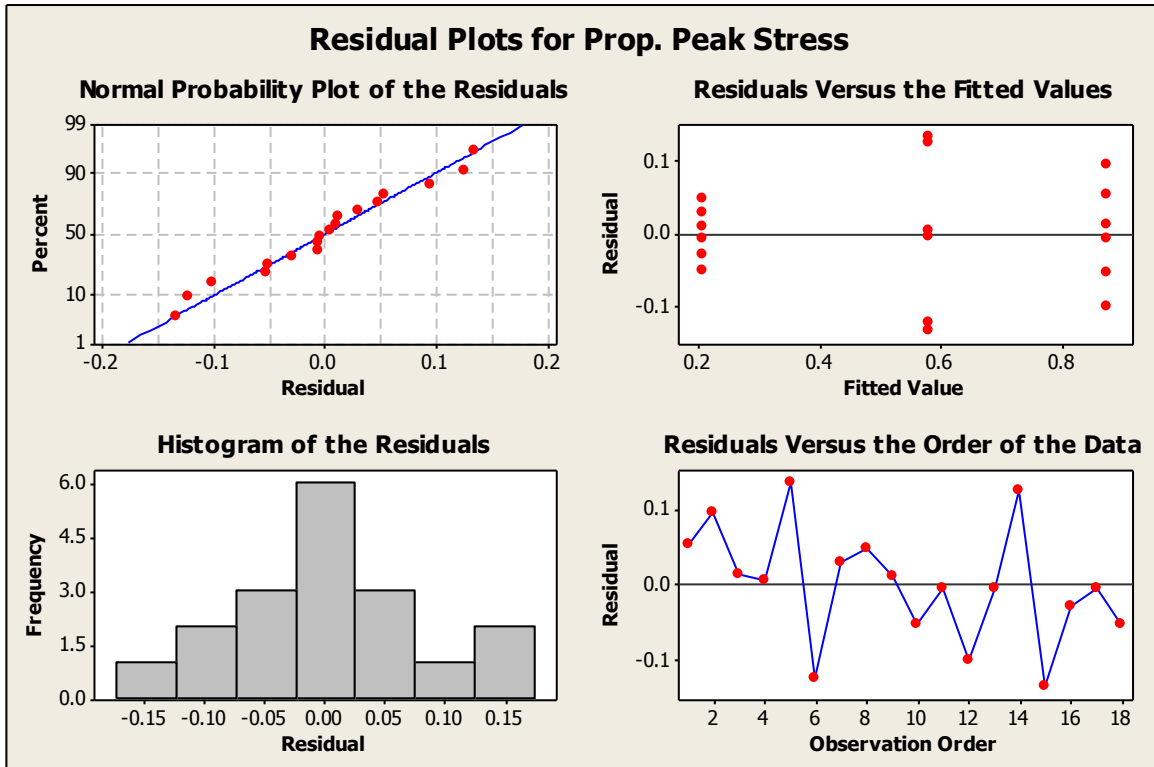


Figure 119: Residual plots for proportional peak stress, material 13

One-way ANOVA: Prop. Peak Stress versus Time for Material 14

Source	DF	SS	MS	F	P
Time	2	0.21132	0.10566	39.93	0.000
Error	15	0.03969	0.00265		
Total	17	0.25101			

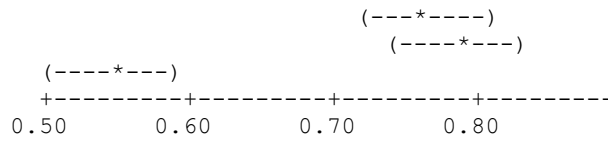
S = 0.05144 R-Sq = 84.19% R-Sq(adj) = 82.08%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev

+-----+-----+-----+-----+-----

2	6	0.76257	0.03602
4	6	0.78662	0.06224
8	6	0.54570	0.05260



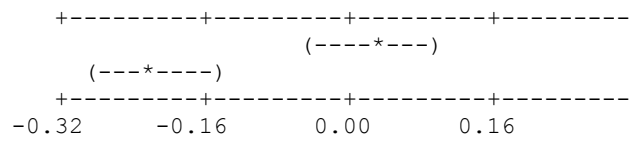
Pooled StDev = 0.05144

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.05302	0.02405	0.10112
8	-0.29395	-0.21688	-0.13981



Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.31800	-0.24093	-0.16385

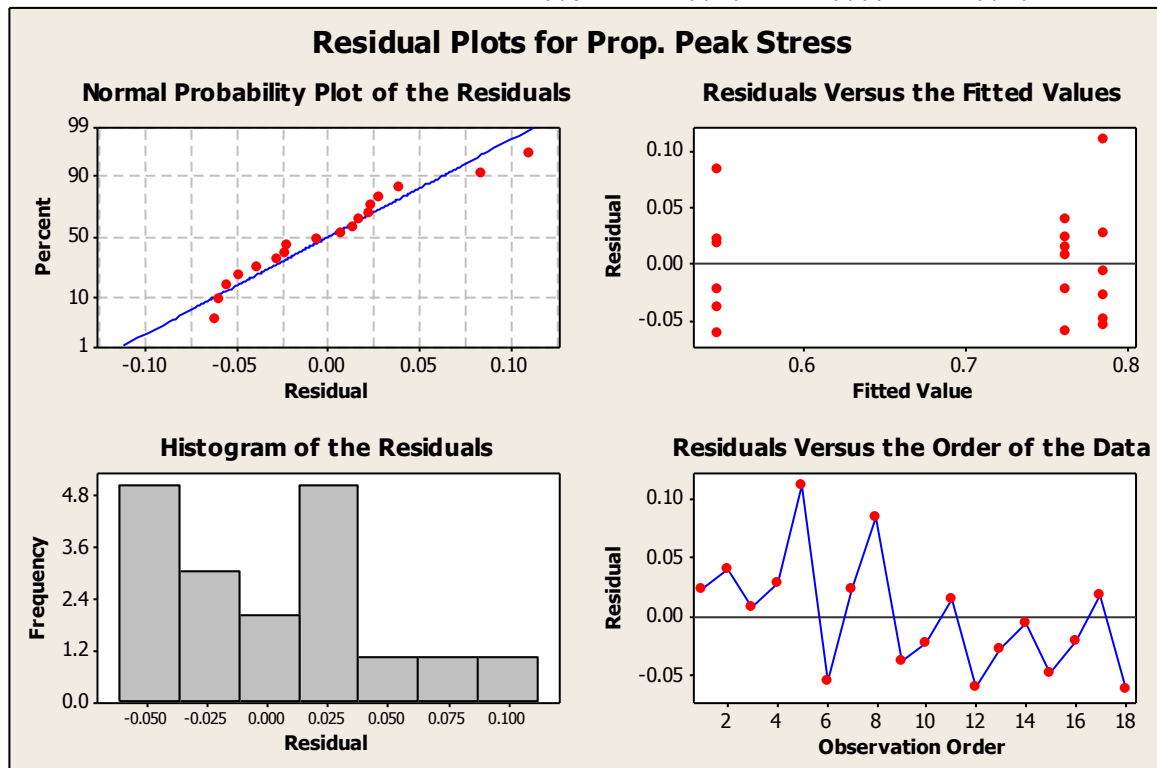
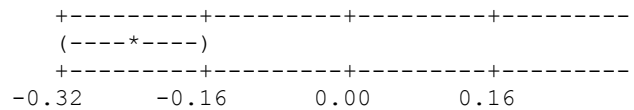
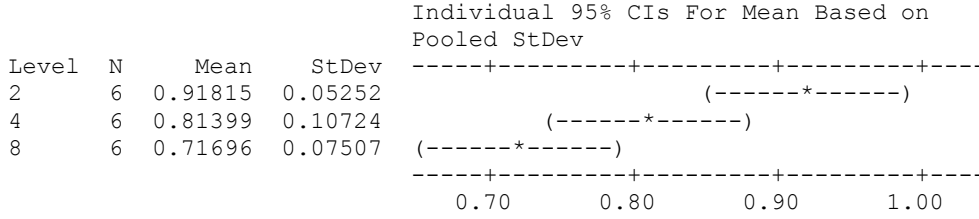


Figure 120: Residual plots for proportional peak stress, material 14

One-way ANOVA: Prop. Peak Stress versus Time for Material 15

Source	DF	SS	MS	F	P
Time	2	0.12148	0.06074	9.16	0.003
Error	15	0.09947	0.00663		
Total	17	0.22095			

S = 0.08143 R-Sq = 54.98% R-Sq(adj) = 48.98%

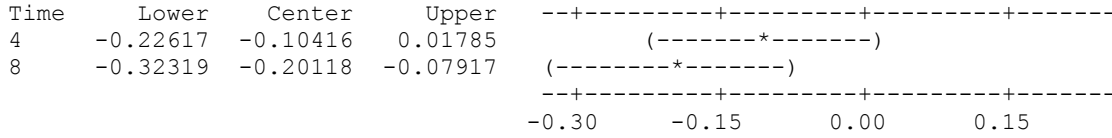


Pooled StDev = 0.08143

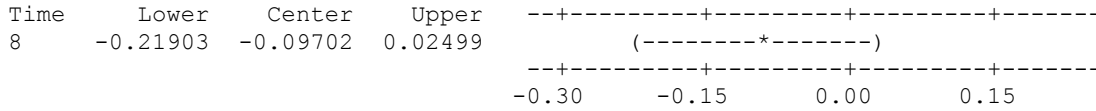
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:



Time = 4 subtracted from:



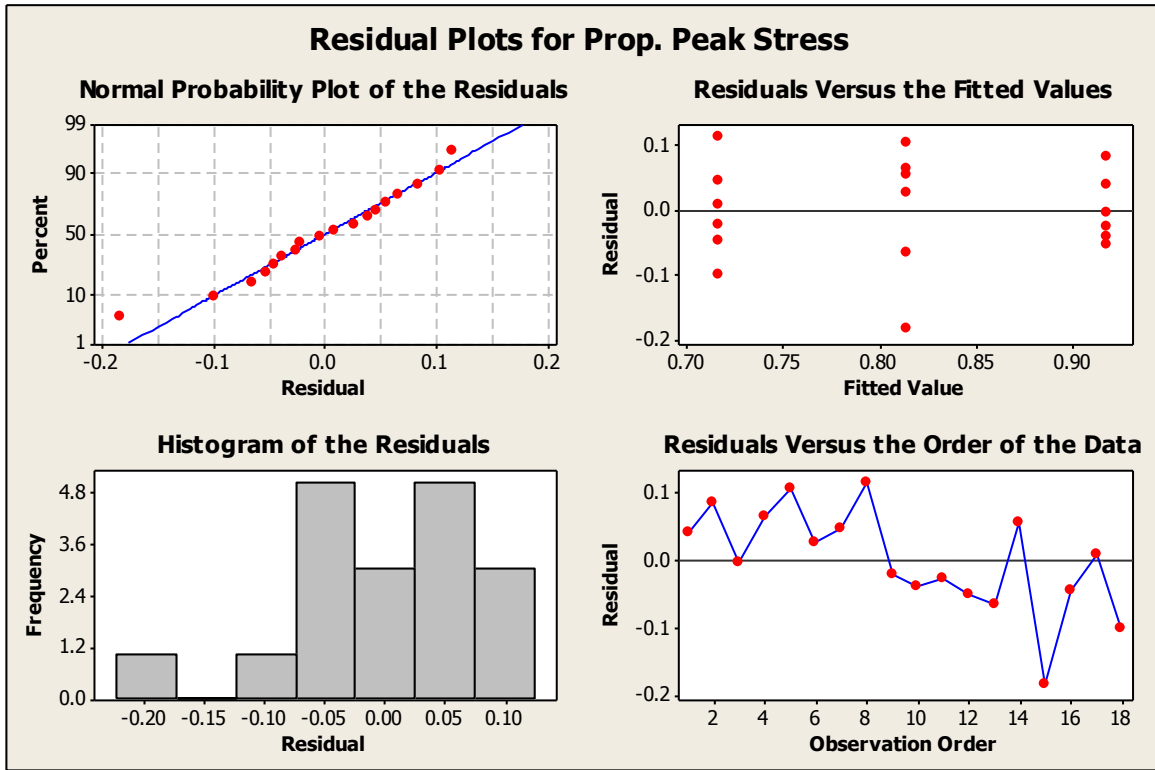
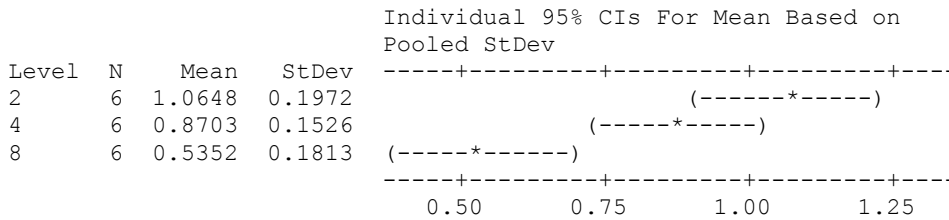


Figure 121: Residual plots for proportional peak stress, material 15

One-way ANOVA: Prop. Peak Stress versus Time for Material 16

Source	DF	SS	MS	F	P
Time	2	0.8612	0.4306	13.59	0.000
Error	15	0.4752	0.0317		
Total	17	1.3363			

S = 0.1780 R-Sq = 64.44% R-Sq(adj) = 59.70%



Pooled StDev = 0.1780

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.4612	-0.1945	0.0721
8	-0.7963	-0.5296	-0.2629

(-----*-----)
(-----*-----)

-0.70 -0.35 0.00 0.35

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.6017	-0.3351	-0.0684

(-----*-----)

-0.70 -0.35 0.00 0.35

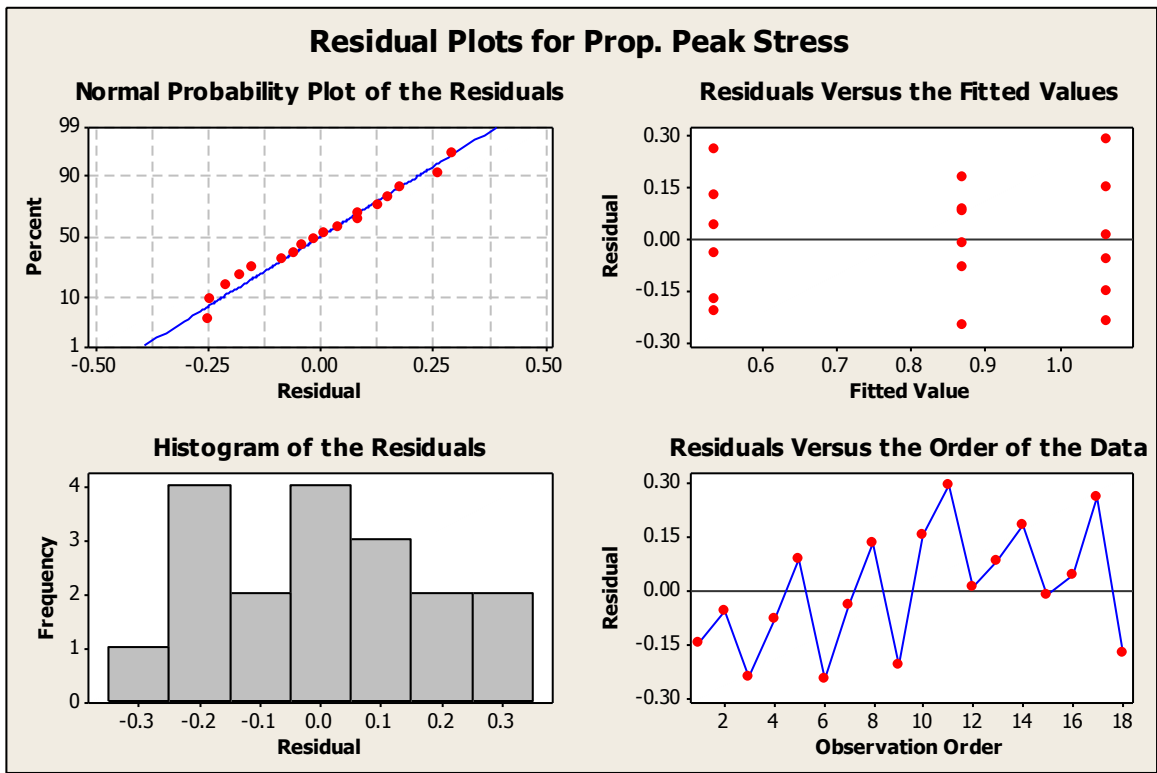


Figure 122: Residual plots for proportional peak stress, material 16

One-way ANOVA: Prop. Peak Stress versus Time for Material 17

Source	DF	SS	MS	F	P
Time	2	0.3661	0.1831	7.37	0.006
Error	15	0.3725	0.0248		
Total	17	0.7387			

S = 0.1576 R-Sq = 49.57% R-Sq(adj) = 42.84%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
2	6	0.9564	0.1342
4	6	0.9005	0.2182
8	6	0.6298	0.0942

Pooled StDev = 0.1576

Tukey 95% Simultaneous Confidence Intervals
 All Pairwise Comparisons among Levels of Time

Individual confidence level = 97.97%

Time = 2 subtracted from:

Time	Lower	Center	Upper
4	-0.2921	-0.0560	0.1802
8	-0.5627	-0.3266	-0.0905

Time = 4 subtracted from:

Time	Lower	Center	Upper
8	-0.5068	-0.2707	-0.0345

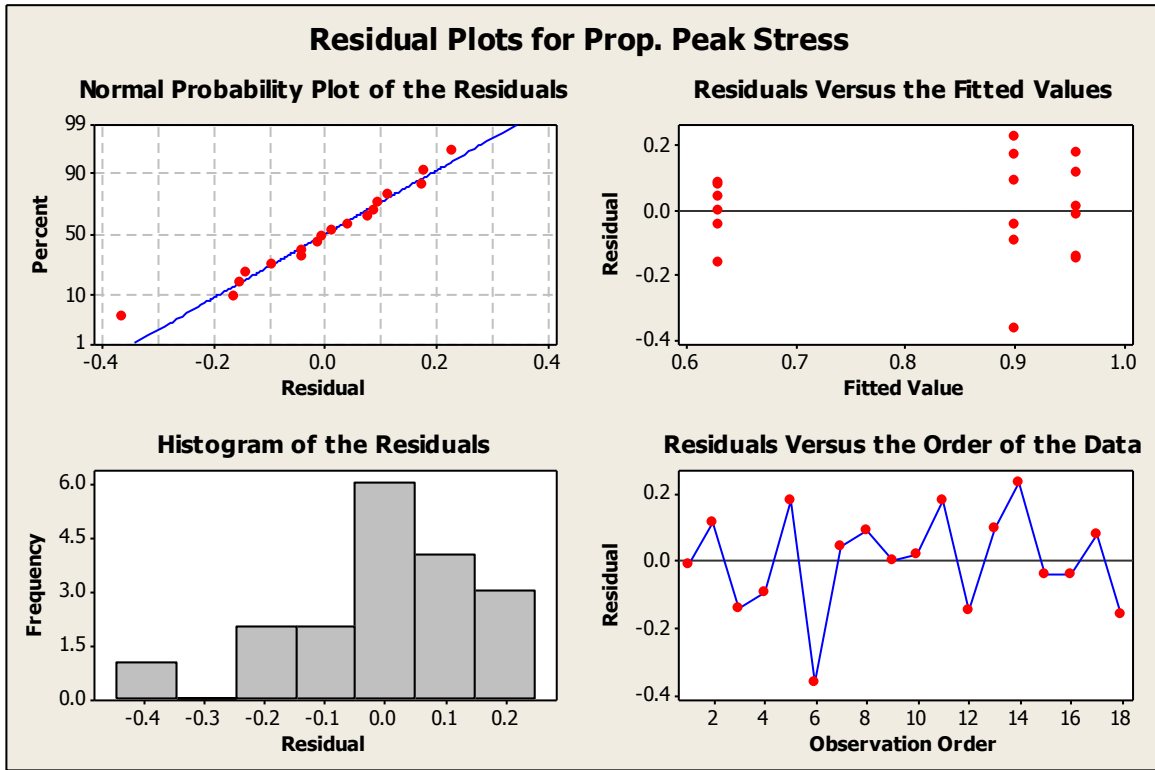


Figure 123: Residual plots for proportional peak stress, material 17