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Andrew Seun
2-yr AEGD Program, Fort Bragg, NC
Uniformed Services University, Bethesda, MD
Date: 01/10/2020

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
POSTGRADUATE DENTAL COLLEGE
SOUTHERN REGION OFFICE
2787 WINFIELD SCOTT ROAD, SUITE 220
JBSA FORT SAM HOUSTON, TEXAS 78234-7510
https://www.usuhs.edu/pdc

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THESIS/MANUSCRIPT APPROVED: DATE:

Erik Reifenstahl *05 June 2020*
Erik Reifenstahl, LTC, DC
ASSISTANT DIRECTOR, AEGD-2, FORT BRAGG
Committee Chairperson

Charles Lambert *05 June 2020*
Charles Lambert, LTC, DC
PROGRAM DIRECTOR, AEGD-2, FORT BRAGG
Committee Member

Manuel Pelaez *05 June 2020*
Manuel Pelaez, LTC, DC
RESEARCH ADVISOR, AEGD-2, FORT BRAGG
Committee Member

EFFECT OF STORAGE TEMPERATURE ON TENSILE BOND STRENGTH OF A SELF-ETCH DENTIN BONDING AGENT

BY

ANDREW SEUN

B.S. University of Illinois at Chicago, 2013
D.M.D. Midwestern University College of Dental Medicine – Illinois, 2017

Submitted for the fulfillment of the requirements
For the degree of Master of Science in the
Department of Oral Biology
In the Graduate School of
The Uniformed Services University of the Health Sciences

FORT BRAGG, NORTH CAROLINA
2020

Submitted by Andrew Seun in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology. Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

Date	Manuel Pelaez, DMD Research Mentor
Date	Erik Reifenstahl, DMD AEGD Assistant Director
Date	Charles Lambert, DDS AEGD Program Director

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DISCLOSURES AND AFFILIATIONS

Neither I, nor any member of my family, have a financial arrangement or affiliation with any corporate organization offering financial support or grant monies for this research, nor do I have a financial interest in any commercial product(s) or service(s) I will discuss in the presentation or publication.

The opinions or assertions contained herein are the private ones of the author(s) and are not to be construed as official or reflecting the view of the DoD or the USUHS. CPT Andrew Seun, DMD, is a resident of the Army Postgraduate Dental School and Uniformed Services University of the Health Sciences Postgraduate Dental College.

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Andrew Seun
2-yr AEGD Program, Fort Bragg, NC
Uniformed Services University, Bethesda, MD

ABSTRACT

Objective: This *in vitro* study investigated the microtensile bond strength (MTBS) of Clearfil SE Bond 2, a self-etch dentin bonding adhesive, at various timepoints at room temperature.

Materials and Methods: 20 non-carious third molars were sectioned mid-coronally to remove all occlusal enamel and expose a flat dentin surface. Teeth were assigned to 3 different groups (n=159) based on adhesive application. Group 1 (Control): Clearfil SE Bond 2 refrigerated (2-8ºC/36-46ºF) per manufacturer's instructions. Group 2 (experimental 1): Clearfil SE Bond 2 stored at room temperature for 1 month. Group 3 (experimental 2): Clearfil SE Bond 2 stored at room temperature for 3 months. Composite resin (3M Filtek Supreme Ultra Shade A2) was added and light-cured incrementally to each tooth to approximately 5mm in height. Teeth were then sectioned for initial microtensile bond strength testing.

Results: A total of 159 samples were tested for tensile bond strength. No between group differences were observed in either mean sample width (0.94 mm, SD = 0.08; P = 0.11) or thickness (0.94 mm, SD = 0.06; P = 0.12). An analysis of variance showed that the effect of storage at room temperature was not significant, P = .066.

Conclusions: No difference in initial tensile bond strengths was observed when storing Clearfil SE Bond 2 in room temperature for 1 and 3 months, or refrigerated per manufacturer's instructions.

INTRODUCTION

Statement of Problem

In the field of modern operative dentistry, the utilization of tooth-colored restorative materials has become well-established and even used exclusively as a direct restorative material in some dental practices. With the higher demand for esthetics, fears associated with amalgam, and the advancements in dental materials, more dentists are placing resin-composite direct restorations than ever before.[1,3] Unlike the amalgam restorative materials used in the past, resin-composites require an adhesive bond for retention to the prepared tooth. For this bond to occur, a complex interaction occurs between the hard tissues of the tooth and adhesive bonding system to retain the resin-composite restorative material.[7] To make these bonds strong and effective, particularly to dentin, has been the challenge since the advent of adhesive dentistry.[19] Over the past few decades, advancements in these special adhesive systems or “dentin-bonding agents” has brought about various degrees of clinical performance.[6] Of the self-adhesive variants of dentin bonding agents, Clearfil SE Bond manufactured by Kuraray (Osaka, Japan) has shown exceptional clinical performance.[4,17,20,21]
One drawback with this product is the storage and pre-application process. The manufacturer of this product instructs users to refrigerate (2-8^{o}C/36-46^{o}F) the product when not in use and to bring the product to room temperature for more than 15 minutes before use.[9] These are two significant drawbacks especially if refrigeration is not available. The wait time before use can also have negative effects in terms of efficiency for completing procedures. The purpose of this study was to investigate the effect of

storage temperature on the initial tensile bond strength of Clearfil SE Bond 2. The potential findings of this study would help inform users using this product the potential drawbacks of not following the manufacturer's instructions for use.

Clearfil SE Bond

Clearfil SE bond is a 6th generation two-step self-etch bonding system with the acidified primer and the adhesive are applied separately.[27] An acidic monomer (primer) is placed and not rinsed. This is a procedural step used to condition and prime the tooth at the same time. The chemical mechanism is via specific carboxyl and phosphate groups of the functional monomers in the primer chemically interacting with the residual hydroxyapatite of the tooth.[2]

Numerous past studies have been conducted evaluating the clinical performance of Clearfil SE bond. One study reported a 98% retention rate in Class V composite restorations at 8 years with and without selective enamel etching of the margins. Separate enamel etching did improve marginal adaptation.[22] Another 8-year non-carious cervical lesion clinical trial showed 97% retention and success rate.[11] A 13-year retention rate study resulted in a 94% retention and success rate.[23]

The bonding effectiveness of Clearfil SE Bond can be attributed to the separation of the acidic monomers in its functional primer from its adhesive agent. Additionally, the adhesive agent contains 10-methacryloxydecyl phosphate (10-MDP). 10-MDP contains phosphate groups capable of producing ionic bonds with calcium in hydroxyapatite. These calcium-phosphate salts tend to be stable without causing strong decalcification.[13,16,17,24,27,28]

Microtensile Bond Strength Test (MTBS)

The microtensile bond strength test (MTBS) is characterized by the use of small beam-shaped specimens with small bonded areas (generally less than 2mm) that generate perpendicular stress to the adhesive interface.[26] Many believe this to be the most reliable method for evaluating bond strengths between an adhesive material and dental substrate due to uniformity in stress distribution.[10] Due to the more uniform stress distribution, the occurrence of cohesive fractures within the tooth material or within the adhesive are greatly reduced. Such failures invalidate the sample by preventing the correct assessment of the interfacial bond. The advantages of this test also include ability to obtain multiple specimens from a single tooth sample.[14]

Differences between Clearfl SE Bond and Clearfil SE Bond 2

According to the manufacturer (Kuraray, Osaka, Japan), Clearfil SE Bond 2 incorporates a new integrated photo-initiator and provides more free radicals when curing. They claim to have higher monomer conversion rates and stronger bonds. Clearfil SE Bond 2 is also compatible with Clearfil DC Activator for use with indirect restorations.[9]

MATERIALS AND METHODS

Tooth Collection/Preparation

Non-carious human teeth indicated for extraction were collected. Specimens were collected immediately after extraction. All remaining soft tissue was removed and specimens were stored in CaviCide by Metrex (Orange, Calfornia, USA) at 21.6-23.3 degrees Celsius (71-74 degrees Fahrenheit). Specimens were prepared as previously described by Cardoso et al., in 2002. Briefly, specimens were stored for a minimum of 24 hours and no more than 8 weeks for disinfection. To test the adhesion of Clearfil SE Bond 2 to dentin, the entire occlusal enamel layer of the specimens was removed. This was accomplished using a water-cooled diamond disc manufactured by Bueler (Lake Bluff, Illinois, USA). To attain a uniform smear layer, the dentinal layer was polished using progressively finer sandpaper (220-,320-,400- grit) manufactured by 3M (Maplewood, Minnesota, USA) for 10 seconds each, followed by a 600-grit for 60 seconds. The bonding surface was washed with water and gently air dried.[8]

Test Groups

- Group 1 (Control – Clearfil SE Bond 2 Refrigerated (2-8^{o}C/36-46^{o}F)
- Group 2 (Test Group – Clearfil SE Bond 2 stored at room temperature (21.6-23.3^{o}C/71-74^{o}F) – 1 month)
- Group 3 (Test Group – Clearfil SE Bond 2 stored at room temperature (21.6-23.3^{o}C/71-74^{o}F) – 3 months)

Application of Adhesive/Composite

Adhesives applied in accordance to manufacturer's instructions.[9] Following adhesive application, a 5x5x5mm composite resin block bonded to adhesive layer.[8] 3M Filek Supreme Ultra Universal Restorative Shade A2 (3M/ESPE, St. Paul, Minnesota, USA) was applied to bonding surface through sequential layered application of 1-2mm in thickness. Each layer cured for 10 seconds at 500nm using a 3M/ESPE Curing light unit. This process was repeated until a final thickness of 5mm was obtained.[14,15]

Sample Sectioning and MTBS Test

Each sample was sectioned and tested based on the following procedure:

1. After bonding and composite placement, each tooth was mounted unto an acrylic jig manufactured by BISCO (Schaumburg, Illinois, USA) to facilitate sectioning.
2. The Isomet 100 manufactured by Buehler (Lake Bluff, Illinois, USA) was utilized at 300rpm with water irrigation. Each tooth was cross and longitudinally sectioned in 1mm increments perpendicular to the adhesive interface with a diamond blade $1mm^2$ beams approximately 10 mm in length
3. Each sample beam was observed to verify that the adhesive interface was perpendicular to the long axis. Samples displaying microfractures were removed. Samples with slanted adhesive interfaces or fractures were also removed.

4. All samples were fixed to a Bisco MTBS testing jig with cyanoacrylate adhesive and tested for MTBS on an Intron 5943 (Norwood, Massachusetts, USA) .
5. Each sample was observed for method of fracture: adhesive, cohesive, dentin, cohesive composite.

Sample Size Calculation

Utilizing the power analysis of GPower V3.1.9.2, the indicated sample size of 159 (53 samples per group) was needed to detect a medium effect (d =.25) at 80% power and with alpha being 0.05.

Statistical Analysis

Exploratory data analyses were conducted on all continuous data (mPa, sample width, and sample thickness). The Shapiro-Wilk test was used to assess the normality of the data distributions. Measures of central tendency are presented as means with associated standard deviations. An analyses of variance (ANOVA) was conducted to check for differences in bond strength (MPa) between the three groups. Statistical significance for all statistical tests was declared at $P < 0.05$. Data were analyzed using SPSS 25.0 (IBM, Armonk, NY, USA).

RESULTS

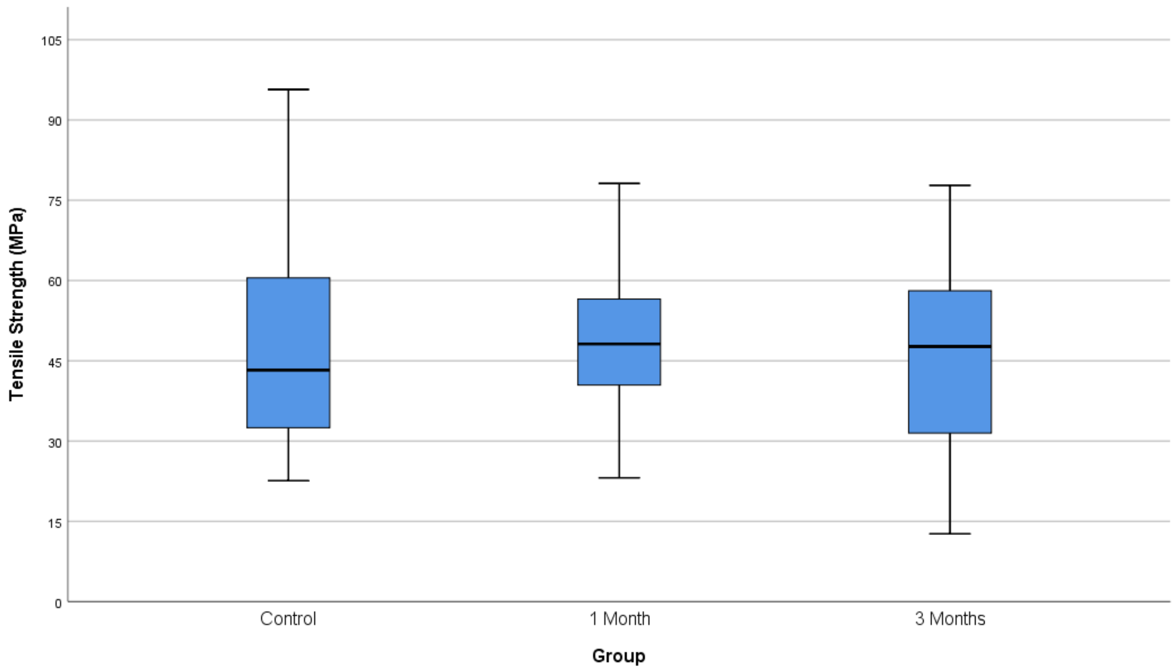
A total of 159 samples were tested for tensile bond strength. No between group differences were observed in either mean sample width (0.94 mm, *SD* = 0.08; *P* = 0.11) or thickness (0.94 mm, *SD* = 0.06; *P* = 0.12). An analysis of variance showed that the effect of storage at room temperature was not significant, *P* = .066. The control samples were found to have a mean tensile bond strength of 48.34 MPa (SD = 19.44). Similarly, after one and three months of storage the mean bond strength of samples was found to be 48.39 MPa (SD = 12.54) and 45.82 MPa (SD = 17.12) respectively.

To check whether the non-significant results were due to a lack of statistical power, we conducted post hoc power analyses based on observed variances with power (1 - β) set at 0.80 and a two-tailed α = 05. This analysis demonstrated that a total sample size of 1920 (640 per group) would have been required in order for group differences to reach statistical significance. Thus, it is unlikely that the lack of difference in tensile bond strength between samples stored for various periods of time can be attributed to a limited sample size.

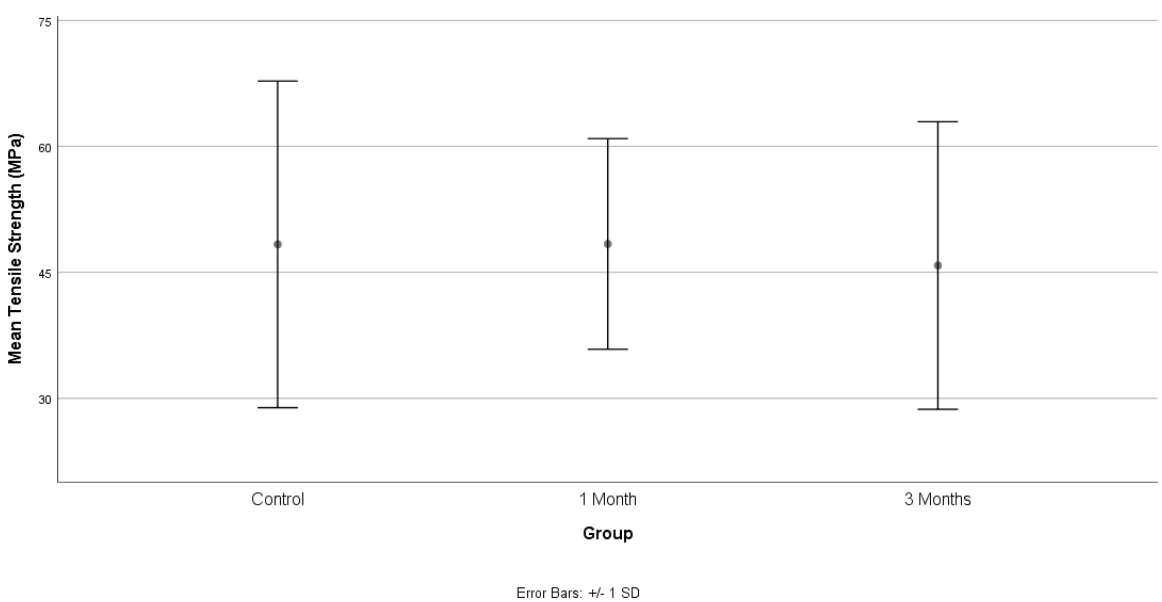
Table 1. Material Characteristics, M(SD)

Characteristic	Control, n=53	1 Month, n=53	3 Month, n=53	P[1]
Bond Strength (MPa)	48.34 (19.44)	48.39 (12.54)	45.82 (17.12)	0.66
Sample width	0.96 (0.11)	0.93 (0.07)	0.94 (0.06)	0.11
Sample Thickness	0.95 (0.07)	0.94 (0.08)	0.92 (0.03)	0.12

1. Significance based on ANOVA.



Tensile Strength (MPa)
105
90
75
60
45
30
15
0
Control
1 Month
3 Months
Group



Mean Tensile Strength (MPa)
75
60
45
30
Control
1 Month
3 Months
Group
Error Bars: +/- 1 SD

DISCUSSION

The results of this study showed no significant difference in initial microtensile bond strength between either the test groups or controls. This was an interesting finding, considering that the manufacturer's instructions for refrigeration were not followed in Group 2 (1 month room temperature storage) and Group 3 (3 month room temperature storage). It was speculated that there would be some breakdown of the components in the product when not refrigerated. Clearfil SE Bond 2 is a self-etch system that contains acidic monomers (MDP), HEMA, and water. Intuitively, the speculation was that prolonged storage in room temperature could affect these specific components. It has been reported in past studies that the hydrolyzation of HEMA to methacrylic acid and ethylene glycol is influenced by storage temperature (27-40^{o}C). This conversion was hypothesized to cause decreases in bond strengths in self-etch bonding systems.[29]

Future studies could investigate the limits of Clearfil SE Bond 2 by extending the storage times. Other variables that could be implemented are variances in temperature and humidity (ie, storing the product in incubators). These parameters would be clinically applicable, as refrigeration is not always available in all clinical settings.

CONCLUSION

Within the confines of this study, it can be concluded that there is no significant difference in initial microtensile bond strengths when storing Clearfil SE Bond 2 at room temperature for 1 and 3 months, or refrigerated per manufacturer's instructions. Further research is needed to investigate if longer storage times would affect results. In addition, other variables such as temperature and humidity variances in addition to storage times would further test the limits of Clearfil SE Bond 2.

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APPENDICES

Table 2: The Ingredients and Manufacturer of Clearfil SE Bond 2 [9]

Adhesive System	Primer (self etching)	Bond (adhesive resin)	Manufacturer
Clearfil SE Bond 2	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), 2-Hydroxyethyl methacrylate (HEMA), Hydrophobic alphatic dimethacrylate, dl-Camphorquinone, Water	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Bisphenol A diglycidylmethacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA), Hydrophobic alphatic dimethacrylate, dl-Camphorquinone, initiators, accelerators, silanated colloidal silica	Kuraray, Osaka, JAPAN

Raw Data

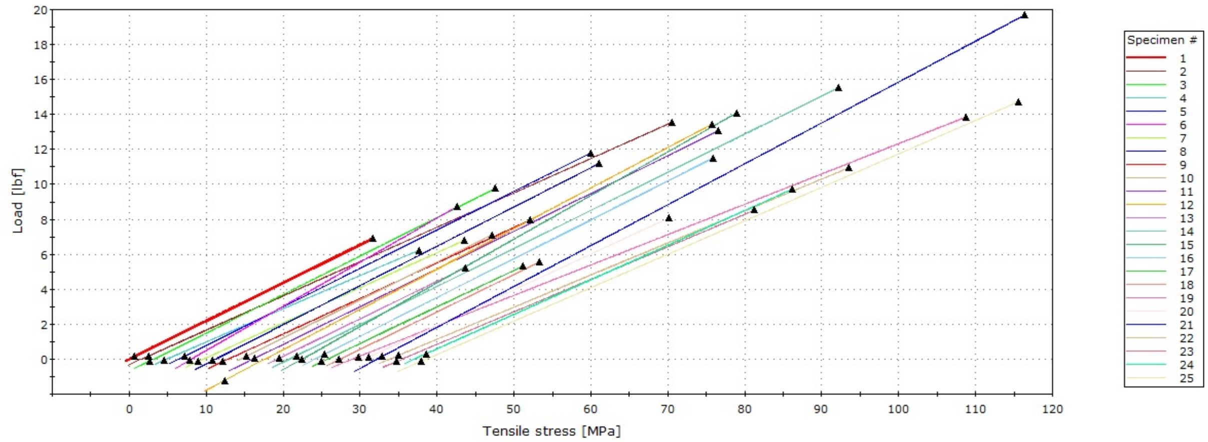
Group 1 (Control)

	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
1	31.65945	0.96000	1.01000
2	68.91055	0.93000	0.94000
3	44.38634	1.02000	0.96000
4	32.82386	0.88000	0.96000
5	53.51629	1.00000	0.98000
6	34.55700	1.06000	1.06000
7	33.95472	0.93000	0.96000
8	49.76808	0.99000	1.01000
9	39.26725	0.94000	0.96000
10	32.68494	0.95000	1.02000
11	60.48017	0.98000	0.98000
12	58.09717	1.00000	1.03000
13	24.35626	0.93000	1.03000
14	71.23212	1.02000	0.95000
15	56.42408	1.10000	1.01000
16	51.70936	0.99000	1.00000
17	25.46314	0.96000	0.97000
18	25.97287	0.98000	0.97000
19	79.74150	1.12000	0.69000
20	39.52089	0.90000	1.01000
21	84.18288	1.00000	1.04000
22	59.72729	0.85000	0.96000
23	45.89394	0.88000	0.94000
24	49.18159	1.00000	0.88000
25	76.90359	0.86000	0.99000

	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
26	55.91108	1.06000	0.86000
27	26.27575	0.93000	0.91000
28	95.71137	0.94000	0.88000
29	29.92221	1.15000	1.01000
30	43.18652	0.89000	1.01000
31	31.19395	1.09000	0.97000
32	51.79832	1.24000	0.94000
33	71.87803	0.75000	0.89000
34	36.93388	0.95000	0.89000
35	64.14296	0.89000	0.90000
36	90.37540	1.01000	0.89000
37	28.54552	1.35000	0.87000
38	32.48134	0.89000	0.86000
39	25.59268	0.87000	1.00000
40	78.45860	0.99000	0.87000
41	24.94262	0.88000	0.93000
42	41.06742	0.88000	0.94000
43	31.96579	0.89000	0.94000
44	43.25268	1.06000	0.91000
45	29.59998	0.95000	0.92000
46	34.00377	0.93000	0.94000
47	57.46114	0.93000	0.70000
48	49.97921	0.96000	0.87000
49	22.59418	0.83000	1.00000
50	39.32397	0.92000	0.88000
51	41.62039	0.80000	0.93000
52	70.52588	0.95000	0.91000
53	27.73623	1.00000	1.00000
Maximum	95.71137	1.35000	1.06000
Mean	47.29993	0.96623	0.94208
Median	43.18652	0.95000	0.95000
Minimum	22.59418	0.75000	0.69000
Standard ...	19.04860	0.10300	0.07231
Mean + 1...	66.34853	1.06922	1.01438
Mean - 1 ...	28.25133	0.86323	0.86977

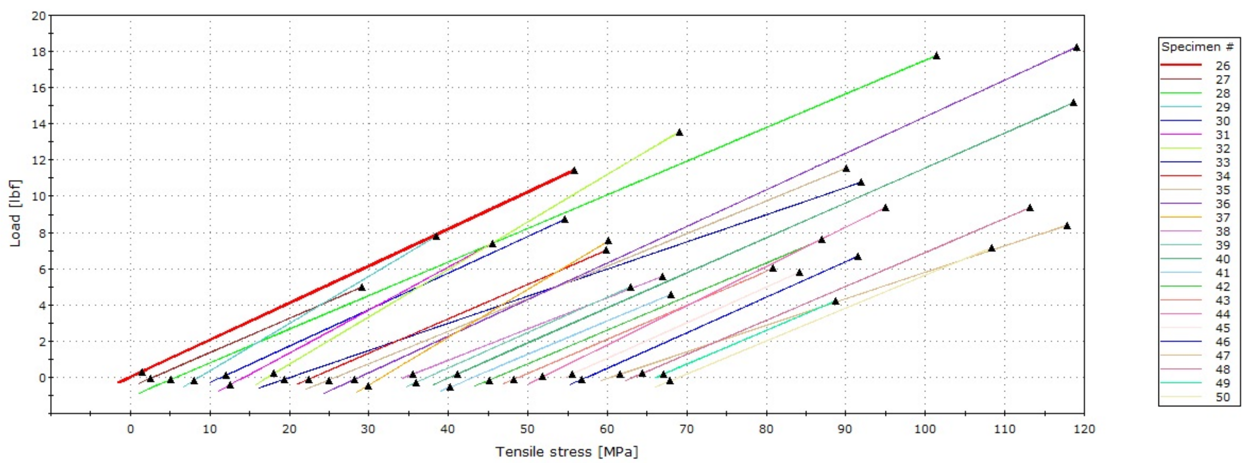
Graph 1

Specimen 1 to 25



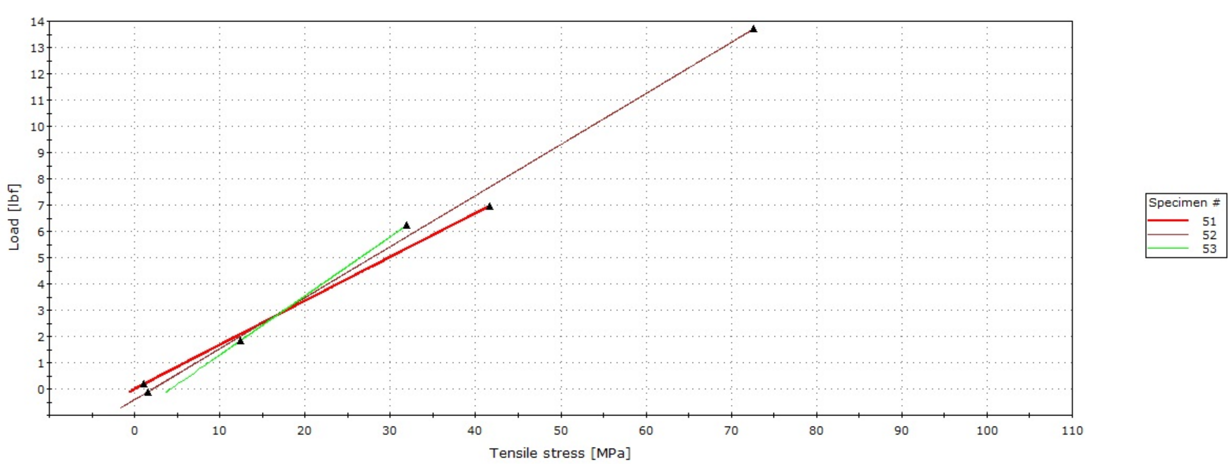
Graph 1

Specimen 26 to 50



Graph 1

Specimen 51 to 53



Group 2 (1 Month)

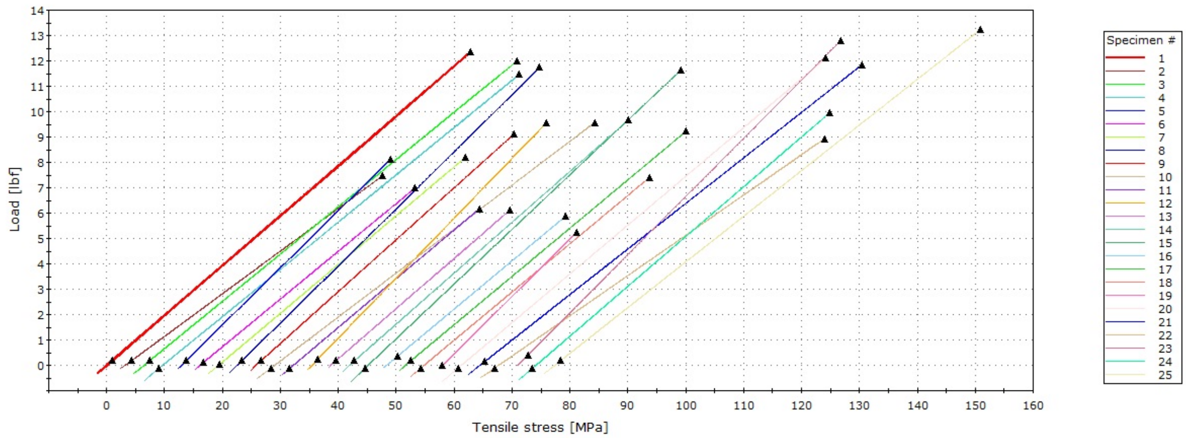
	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
1	62.86890	0.91000	0.96000
2	44.47956	0.86000	0.87000
3	64.38032	0.92000	0.90000
4	61.65140	0.88000	0.94000
5	36.18906	1.00000	1.00000
6	37.16431	0.92000	0.91000
7	42.69640	0.93000	0.92000
8	52.24549	1.00000	1.00000
9	44.57500	0.91000	1.00000
10	55.25086	0.94000	0.82000
11	32.10651	0.91000	0.94000
12	40.45412	0.93000	1.13000
13	30.90154	0.83000	1.06000
14	48.13993	0.93000	0.96000
15	54.10676	0.93000	1.03000
16	30.87016	0.91000	0.93000
17	48.52609	0.92000	0.92000
18	38.93152	0.94000	0.90000
19	23.12988	1.04000	0.97000
20	62.99083	0.90000	0.95000
21	66.01601	0.94000	0.85000
22	56.30465	0.84000	0.84000
23	55.91540	1.04000	0.98000
24	50.71185	0.91000	0.96000
25	73.61853	0.91000	0.88000

	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
26	78.15352	0.88000	0.89000
27	56.72525	0.92000	0.91000
28	31.99425	1.20000	0.90000
29	46.05435	0.92000	0.90000
30	49.91456	0.96000	0.95000
31	43.22063	0.86000	0.88000
32	30.68690	1.02000	1.10000
33	47.07292	1.00000	0.94000
34	62.56794	0.89000	0.87000
35	42.16529	1.00000	1.00000
36	42.49695	1.00000	1.00000
37	53.31072	0.93000	0.92000
38	63.93426	1.00000	1.00000
39	62.02729	1.00000	1.00000
40	33.50338	1.00000	1.00000
41	48.83540	1.00000	1.00000
42	30.10695	1.00000	1.00000
43	41.01397	1.00000	1.00000
44	50.46677	1.00000	1.00000
45	37.35513	1.00000	1.00000
46	55.21026	1.00000	1.00000
47	46.05923	1.00000	1.00000
48	56.53046	1.00000	1.00000
49	27.60762	1.00000	1.00000
50	42.74597	1.00000	1.00000
51	46.47390	1.00000	1.00000
52	57.99819	1.00000	1.00000
53	69.24341	0.90000	0.92000

Maximum	78.15352	1.20000	1.13000
Mean	48.44718	0.95528	0.95849
Median	48.13993	0.94000	0.96000
Minimum	23.12988	0.83000	0.82000
Standard ...	12.53903	0.06351	0.06262
Mean + 1...	60.98621	1.01879	1.02111
Mean - 1 ...	35.90815	0.89178	0.89587

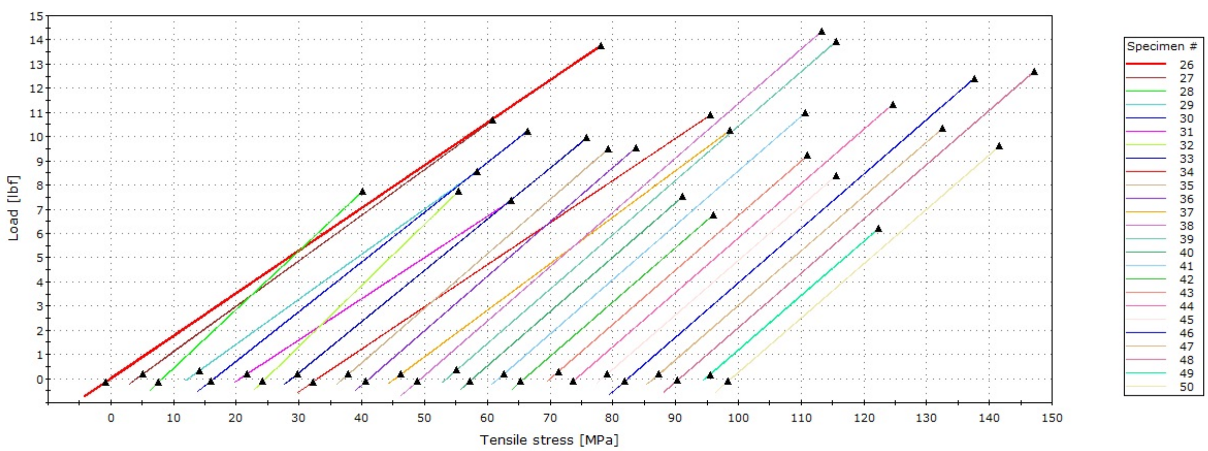
Graph 1

Specimen 1 to 25

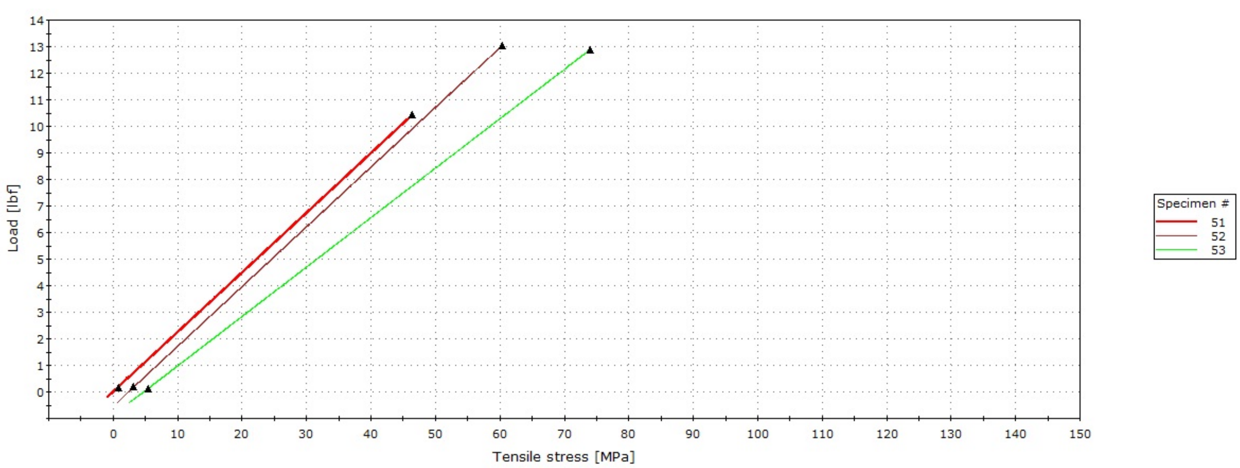


Graph 1

Specimen 26 to 50



Specimen 51 to 53



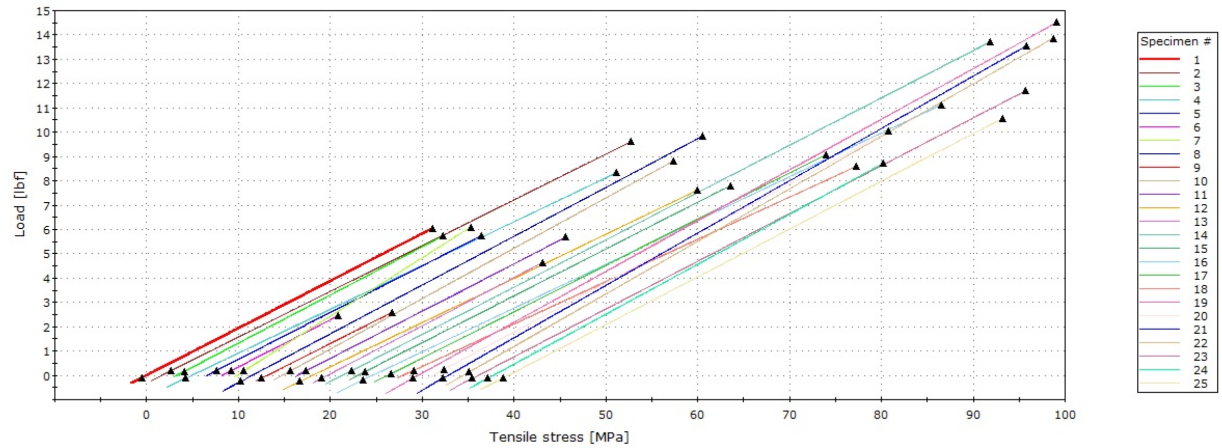
Group 3 (3 Months)

	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
1	31.13323	0.89000	0.97000
2	51.07783	0.93000	0.90000
3	28.99981	1.00000	0.88000
4	46.24432	0.90000	0.89000
5	29.90361	0.89000	0.96000
6	12.67673	0.98000	0.88000
7	25.43398	1.16000	0.92000
8	49.09397	0.92000	0.97000
9	13.60207	0.92000	0.92000
10	42.61234	0.93000	0.99000
11	29.21026	0.94000	0.92000
12	41.86716	0.94000	0.86000
13	23.39441	0.90000	0.98000
14	70.55627	0.92000	0.94000
15	40.59383	0.93000	0.92000
16	61.86853	0.91000	0.88000
17	47.67530	0.92000	0.92000
18	49.33374	0.88000	0.88000
19	69.59174	0.91000	1.02000
20	49.63842	0.98000	0.92000
21	62.95024	1.03000	0.93000
22	64.23239	0.97000	0.99000
23	59.61455	0.96000	0.91000
24	42.43871	1.05000	0.87000
25	53.81969	0.95000	0.92000

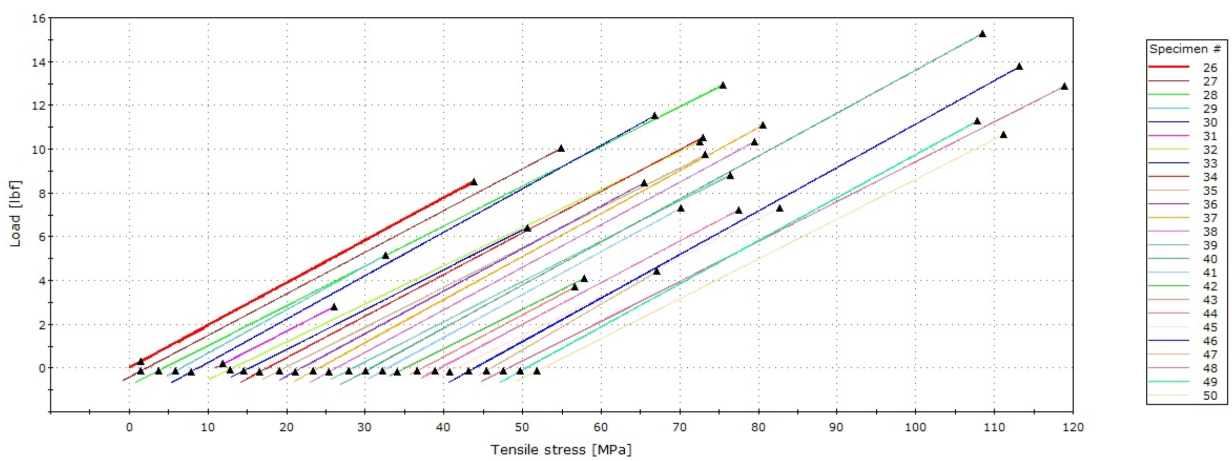
	Tensile stress at Maximum Load [MPa]	Width [mm]	Thickness [mm]
26	43.81463	0.95000	0.91000
27	52.74617	0.92000	0.92000
28	71.09798	0.89000	0.91000
29	26.05063	0.92000	0.96000
30	58.08242	0.96000	0.92000
31	15.09012	0.94000	0.88000
32	59.44698	0.86000	0.90000
33	35.25573	0.92000	0.88000
34	55.36788	0.94000	0.90000
35	53.49924	0.92000	0.88000
36	43.60528	0.94000	0.92000
37	56.42792	0.92000	0.95000
38	53.21501	0.91000	0.95000
39	47.88862	0.91000	0.90000
40	77.77977	0.91000	0.96000
41	37.24116	0.97000	0.90000
42	22.79155	0.87000	0.92000
43	19.40548	0.92000	0.92000
44	38.02632	0.88000	0.96000
45	41.04024	0.88000	0.90000
46	69.36671	0.96000	0.92000
47	21.06452	0.97000	0.96000
48	70.72128	0.91000	0.89000
49	57.43829	0.94000	0.93000
50	58.60134	0.88000	0.92000
51	77.58658	0.88000	0.90000
52	31.48026	0.95000	0.90000
53	41.53708	1.09000	0.95000

Maximum	77.77977	1.16000	1.02000
Mean	45.91004	0.93623	0.92170
Median	47.67530	0.92000	0.92000
Minimum	12.67673	0.86000	0.86000
Standard ...	17.01977	0.05390	0.03457
Mean + 1...	62.92982	0.99012	0.95627
Mean - 1 ...	28.89027	0.88233	0.88713

Specimen 1 to 25



Specimen 26 to 50



Specimen 51 to 53