Title

A Standardized Characterization of the Mechanical and Thermal Properties of Orthodontic Clear Aligners

Author

Li, Fangming

Publication Date

2020

UNIVERSITY OF CALIFORNIA

Los Angeles

A Standardized Characterization of the Mechanical and Thermal Properties of Orthodontic Clear Aligners

A thesis submitted in partial satisfaction of the
requirements for the degree of Master of Science
in Oral Biology

by

Fangming Li

2020

ABSTRACT OF THE THESIS

A Standardized Characterization of the Mechanical and Thermal Properties of Orthodontic Clear Aligners

by

Fangming Li

Master of Science in Oral Biology

University of California, Los Angeles, 2020

Professor Benjamin Wu, Chair

Clear aligners are becoming a popular mode of treatment in orthodontics. Despite their increase in use and demand, there is a general lack of literature regarding the fundamental intrinsic mechanical properties of clear aligners. Therefore, it becomes difficult for patients and clinicians to compare the performances of different aligner products. Existing studies have investigated the biomechanical properties of raw plastic sheets used to make aligners, and sheets thermoformed over simple geometries (i.e. standard blocks). However, the results obtained from standard blocks have limited clinical significance. Instead, aligner samples with actual dental morphology need to be tested to yield clinically meaningful data. Therefore, the aim of this study is twofold. First, we set forth a standardized protocol to characterize significant properties of both raw thermoplastic sheets and fabricated aligners. Second, we evaluated the effects of processing and intraoral components on the aligner materials. These components include heat treatment during aligner manufacturing processes, water soaking, and pH alternations.

To characterize the mechanical properties of the thermoplastic sheets and fabricated aligners, we first characterized the structural compositions of aligners by measuring the degree of crystallinity of each material. Second, we used TA Instruments® Dynamic Mechanical Analyzer (DMA) to quantify critical properties such as stress relaxation and creep of aligner samples with high accuracy and precision. Third, we compared strength and resistance from macroscopic and microscopic levels to analyze crack resistance and microhardness. We also measured Arrhenius activation energy and glass transition temperature to study physical phase transition and molecular interactions after thermomechanical changes. Moreover, we mapped out thickness distributions and quantified the light transmission of different aligner materials. Lastly, we examined the effects of heat treatment and water-polymer interactions on the aforementioned mechanical properties.

Our findings suggest that thermoforming processes reduced aligner thickness and stiffness, increased hydrophilicity and water absorption, modified crack resistance, transparency, glass transitioning temperature, and microhardness in some materials. Water soaking resulted in reduced initial stress and Arrhenius energy, but increased stress relaxation and creep compliance compared to the unsoaked controls. Aligners with different chemical compositions exhibited distinct mechanical characteristics and responded differently to the changes in heat treatment and water soaking. In summary, we found that heat treatment and water immersion can lead to significant degradation of various important mechanical and thermal properties of aligners. Thus, the clinical performances of aligners may be affected by thermoprocessing and intraoral use.

The long-term goal is to share our protocols with other independent institutions so that we can create collaborative monitoring and analysis of all aligner products in the market. The clinical impact is to improve patient care by allowing clinicians to make evidence-based decisions in material selection.

The thesis of Fangming Li is approved.

Alireza Moshaverinia

Ichiro Nishimura

Min Lee

Benjamin Wu, Committee Chair

University of California, Los Angeles

2020

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Professor Benjamin Wu and Dr. Yulong Zhang for their mentorship, guidance and continuous support throughout my education. Their passion, dedication and work ethics are truly inspirational to me.

In addition, I would like to thank all other lab members, Dr. Giovanny Acosta, Dr. Yun Chang Lee and Dr. Faheem Amir Soalngi for their wonderful suggestions and feedbacks for my presentation. Many thanks to Professor Chase Linsley for his tremendous help in my thesis revision.

I am honored to have Professor Alireza Moshaverinia, Professor Ichiro Nishimura and Professor Min Lee for being my committee members. I am very grateful for their valuable suggestions and recommendations.

Many thanks to Mr. Matthew Dingman in the Oral Biology department for his assistance in administrative work.

I would also like to thank my parents, Jenny Jeon, Victor Dane, Carolina Torres, Diana Velicu, Michael Hay, Brian Mao, Wumian Zhao and James Han for their support throughout my residency and Masters in Oral Biology.

Finally, I would like to acknowledge the American Association Orthodontics Foundation Center Award for funding my project.

I. INTRODUCTION

Orthodontic clear aligners are becoming an increasingly popular mode of treatment as alternative to conventional fixed appliances in correcting mild to moderate dental malocclusions.[1] Aligners are accepted by patients as a more esthetic and comfortable treatment alternative compared to traditional braces.[2] In North America, approximately 10% of the 3 million new orthodontic patients were treated with clear orthodontic aligners in 2016.[3] As such, the global aligner market was projected to grow at a compound annual growth rate of 12.68 % from 2016-2020.[4]

Upon the expiry of Align Technology (Invisalign®) patents, more manufacturers, commercial labs and aligner companies have emerged in the market. These aligner providers are marketing their aligner products based on different materials, scanning technologies, set-up software, thickness, transparency and finishing quality, claiming to provide satisfactory treatment outcomes.[5] However, there is a general lack of quality data that compares the performance of different aligners, making it difficult for comparisons to be made between different aligner products.

Compared to traditional fixed braces, the clinical results of aligner therapies may be less predictable. [6] Aligners generate tooth movements through a series of trays, with each tray designed to produce progressive incremental movement of 0.1 to 1.0 mm depending on the manufacture.[7] However, the tooth movements observed clinically frequently do not align with the designed outcomes. As high as 70% to 80% of clear aligner cases required midcourse correction, case refinement, or conversion to fixed appliances before the end of treatment.[1,8] During the course of the treatment, the discrepancies between teeth and aligner trays may become sufficiently large so that aligners would no longer adapt to tooth surfaces well enough to produce the designated movements.[9] It is reported that certain orthodontic movements such as extrusion and rotation of canines and premolars only have 30-40% of clinical accuracy.[6,9] Clinicians often have to overprescribe the amount of movements during planning stages to compensate for the anticipated inaccuracies to achieve the desirable results.[10] As a result, aligners are not widely used for more complex cases that require precise control of root angulations, such as cases that involve tooth extractions or large anteroposterior dental discrepancies, as the results may be less predictable.[6,11,12] Patient compliance, staging strategy, 3D model printing accuracy and orthodontic mechanics can partially contribute to the compromised treatment outcomes of aligner therapies. However, more recent studies have attributed the intrinsic mechanical properties of aligners for their lackluster clinical results and limited applicability to treat complex cases.[7,13-24]

The thermoplastic materials used in removable aligners exhibit very different mechanical properties compared to the metal wires used in fixed orthodontics.[15,22] For example, nickel titanium (NiTi) wires used in fixed orthodontic techniques can exert constant forces over a range of deflections. In other words, NiTi wires can produce continuous forces throughout the designated range of orthodontic tooth movements.[15,22] Clear aligner materials, on the other hand, are viscoelastic, exhibiting intermediate properties between viscous and elastic materials.[25] The mechanical performance of aligners begin to decay immediately upon intraoral insertion well before any tooth movements are achieved.[14,16,26] Specifically, two key mechanical properties, stress relaxation and creep, underlie the performance degradation of viscoelastic materials.[15,26] When aligners are stretched with repeated use or compressed by occlusal forces below the yield point, permanent deformation occurs slowly, a property known as creep.[25] Over time, aligners can slowly deform as teeth are moved into position,[18] and this deformation, or creep, can compromise clinical outcome as aligners may no longer adapt well to the dentition.[15]

In addition, the force that aligners exert deteriorates considerably over time, another unique property of viscoelastic aligner material known as stress relaxation[15,16,27] . Li et al. showed that rapid stress relaxation of clear aligners occurs in the first 24 hours of testing, and the residual orthodontic force continues to decrease and plateaus by the fourth day.[28] As a result, aligners may only deliver 60-70% of force required for tooth movement after stress relaxation,[6] a stark contrast to NiTi wires that exert continuous forces necessary for tooth movement.[16]

Furthermore, there is a large variation of mechanical properties among different aligner products based on their chemical compositions. Currently, a wide range of materials are utilized for different aligner brands. The most commonly used aligner materials include polyethylene terephthalate (PET)[29], modified PETG (polyethylene terephthalate glycol)[29], copolyester[2], polypropylene[2], polycarbonate[2], polyvinyl chloride[30], polyurethane (PU)[2,31], polypropylene (PP) and ethylene vinyl acetate (EVA)[5]. Each aligner material has distinct structures and level of crystallinity. Depending on the degree of crystallinity, the structures of aligner materials can range from amorphous (i.e. no crystals present) to crystalline. The amount of crystalline structures within the materials may greatly dictate the elasticity, impact resistance, brittleness and strength of materials. In addition, the molecular sizes, weight and chain orientation vary greatly between materials. For example, it is reported that polyurethane and polyester materials have disparate resistance to creep.[15] In addition, stress relaxation is also found to be greatly affected by material compositions. Stress relaxation of PETG and polyurethane materials can range from 15% to 44%.[16] Collectively, the different chemical and physical makeup of aligner materials contribute to the unique optical, mechanical, thermal and chemical properties of each plastic material.[5,8,20,25,32-34]

However, the exact compositions of different aligner materials are not readily available as they are proprietary to each manufacturer. Therefore, it is difficult to predict the mechanical properties and performances of aligner materials by extrapolating from existing data as the compositions are mostly unknown to the public. Thus, there is a need to directly test these materials as we do not fully know their composition.

Despite the need to test these materials, there is a dearth of standardized testing protocols to investigate the intrinsic properties of aligners. Most of the existing studies have tested raw material or thermoplastic sheets rather than fabricated aligners that are used clinically, partially because mechanical testers often require a large sample dimension. For example, the Instron™ system is one of the most popular devices that characterizes key properties such as stress relaxation and creep.[35] It requires a flat sample surface and a length of 40-90 mm (Fig 1, A).[14,26,35] In other testing systems, rectangular specimens with a dimension of 5 mm in width and 40 mm in length are required for tensile force tests.[24] However, the sample dimensions that can be obtained from aligners are much smaller than what is required by these testing systems (Fig 1, B). Therefore, there is a need to explore new testing systems that can measure properties of small aligner samples with high reliability and accuracy.

Moreover, there is a lack of consistency in terms of the testing methods in current literature, which makes comparison of aligners properties difficult. For example, some studies used three-point bending tests that utilize compressive forces to characterize the force delivery of aligner materials[7,8,14,16,20,21,26,36,37], while other studies employed two-point stretching method that uses tensile forces to generate these results[13,24,26]. It is difficult to compare between the results obtained from compressive and tensile forces and draw meaningful clinical conclusions. In fact, a material that undergoes flexural testing has both localized tensile and compressive zones on the same sample.[25] Therefore, there is a need to develop standardized protocols that characterize the properties of both tensile and compressive zones present in the same aligner sample.

The molecular and structural orientation may also differ within the same aligner material depending on the manufacturing processes, conferring different physical properties within the aligner material.[24] As a result, aligner materials can be anisotropic, meaning that a physical property can have different values when measured in different directions.[38,39] For example, elastic modulus measured from directions that are parallel to molecular orientations is higher than that resulted from orthogonal orientations. [24,38,39] Therefore, there is a need to develop standardized testing protocols in order to compare results across studies.

In conjunction to inconsistencies in testing protocols, findings in existing literature offer limited clinical applications. Instead of testing aligners that are thermoformed on models of teeth, most of the materials tested in previous studies were made by impressing onto a stone block with standardized dimensions.[7,19,24] The advantage of using aligners formed on standardized blocks is that it simplifies the experiential design by producing a flat and homogenized sample for testing.[24] However, the aligners used clinically have curvatures that follow the morphology of teeth,[7] and the complex dental geometrical form may significantly alter the local mechanical properties of the materials.[24,40] Therefore, the results obtained from standard blocks have limited clinical implications; instead, aligner samples with actual dental morphology should be tested for clinically meaningful data.

To tackle these problems, we developed a series of protocols for standardized characterization of key properties of both raw aligner sheets and aligners thermoformed over patient dental models. The important mechanical and thermal properties that were selected in this study include crystallinity, glass transition temperature, stress relaxation, creep, microhardness, crack resistance, light transmittance, Arrhenius energy, hydrophilicity, water absorption, and aligner thickness distribution. First, we used Differential Scanning Calorimetry (DSC) to characterize the crystallinity of different aligner materials. Second, we utilized Dynamic Mechanical Analyzer (DMA) to quantify critical properties such as stress relaxation and creep of original sheets and fabricated aligner samples with high accuracy and precision.[41] Third, we developed protocols to analyze crack resistance and microhardness with Vickers indenter and impact tester, allowing us to compare strength and resistance from macroscopic and microscopic levels. Fourth, we studied light transmittance with light spectrophotometer to understand the optical properties of the plastic materials. From the thermodynamic and energetics perspectives, we measured Arrhenius activation energy and glass transition temperature with DMA to study physical phase transition and molecular interactions to predict thermomechanical changes and justify the observed mechanical properties in our findings. In addition, we mapped out the topographical distributions of thickness in different aligners. We also quantified hydrophilicity of the materials with Kruss DSA 100. Lastly, we investigated the effects of heat treatment and polymer-water interactions on the aforementioned mechanical properties.

The materials tested in the study include PETG (polyethylene terephthalate glycol) and TPU (thermoplastic polyurethane) materials proprietary to Angelalign™ and Invisalign™ aligner companies. PETG material is widely used by aligner companies or dental offices for aligner fabrication.[13,14] TPU materials are the most popular material used by some of the biggest aligner manufacturers.[24]

With these new protocols, we aim to investigate how the mechanical properties of these most popular aligner materials are altered from fabrication processes to clinical use.

The alterations that the raw thermoplastic material undergo from fabrication to clinical use are vast. During fabrication, flat raw materials sheets are softened by heat so that they can be impressed onto dental models to conform to the anatomy of teeth to form aligner trays.[19] The heat treatment processes were found to reduce the thickness and transparency, increase water absorption, alter surface hardness and flexural strength of plastics materials in previous studies.[7,19] The effects of heat treatment on aligner materials used in clinical settings will be investigated in this study.

In addition, intraoral environments may alter the thermomechanical properties of aligners during clinical use.[13] Water absorption as well as interactions with ions, enzymes, bacterial byproducts, and ingested components and change in pH may lead to reversible or irreversible changes in polymeric structures and mechanical properties,[5,18,42,43] including glass transition temperature and strength.[24,38] Ryokawa et al. reported a correlation between increased water absorption and reduction of tensile strength in eight thermoplastic sheet materials.[24] Oral temperature may play a role in the thermo-aging process of aligners.[3,10,13,18,26] The previous studies tested original thermoplastic sheets, but there is a lack of studies that test the effects of water and pH on fabricated aligners. In our study, we will evaluate how water modifies thermomechanical properties of aligner materials.

Ultimately, the long-term goal of this project is to establish a data repository to share protocols and data with institutions around the world for collaborative monitoring and analysis of all available commercial aligner materials. Ultimately, this endeavor will improve patient care by informing clinicians with evidence-based material selection.

II. OBJECTIVES AND SPECIFIC AIMS

The objective of this project was to develop new protocols to characterize key mechanical properties of clear aligners. We evaluated the effects of heat treatment, water absorption and low pH on the properties. The long-term goal is to promote patient care by informing clinicians and patients with evidence-based material selection.

SPECIFIC AIMS

AIM 1: Develop testing protocols and characterize key properties of aligners, including crystallinity characterization, glass transition temperature, microhardness, crack resistance, light transmittance, stress relaxation, creep and Arrhenius energy.

AIM 2: Evaluate the effects of heat treatment during of aligner manufacturing processes on thermomechanical properties of aligner materials.

AIM 3: Investigate the effects of water absorption and low pH on thermomechanical properties of aligner materials.

III. MATERIALS AND METHODS

III a. Clarification of Nomenclature

Raw or original materials: original thermoplastic sheets before thermoforming processes (i.e., heat treatment) or soaking in water or a pH medium.

Thermoformed or heat-treated aligner materials: original sheets that were treated with heat to form aligners by impressing onto standardized dental models with a vacuum forming machine.

Soaked materials: materials (either raw or thermoformed) that were soaked in either PBS or low pH medium at 37° C for different time intervals.

III b. Materials and Sample Preparation

Aligner Materials

Three materials were tested in the current study, namely PETG (polyethylene terephthalate glycol), TPU-1 (thermoplastic polyurethane-1), and TPU-2 (thermoplastic polyurethane-2). PETG is a widely used material used by aligner companies and clinicians to fabricate commercial or in-house aligners.[13] TPU-1 was the aligner material used by Angelalign™ company. TPU-2 material was obtained by Invisalign™. Both raw and thermoformed materials were tested in this study (Table I). The tested raw material sheets include PETG and TPU-1. Both raw materials had 0.76 mm in thickness. We did not include TPU-2 raw material in our study as the raw material is proprietary to Align Technology and it is not available to the public. However, it is reported that the raw material of TPU-2 was also 0.76 mm in thickness.[24] Therefore the thickesss of the three aligner raw materials were standardized. The tested thermoformed materials include aligners fabricated from PETG, TPU-1, as well as aligners purchased from Invisalign™ (TPU-2) which were tested as received. All thermoformed aligners (PETG, TPU-1 and TPU-2) were made from the same set of patient models.

Heat Treatment in Thermoforming Processes

Raw material sheets of PETG, TPU-1 were subject to heat treatment to form aligners. The flat original sheets were mounted in thermoforming unit (Biostar®; Scheu Dental). They were placed beneath the heating unit and heated to a suitable temperature recommended by the manufacturers until materials softened and began to sag slightly.[19] The softened thermoformable sheets were impressed onto the same set of dental model casts under vacuum suction. The clear aligners were trimmed to dentogingval margins.

Water Soakage

Part of raw and thermoformed material samples were of soaked in PBS (pH = 7.4) for 1, 2, 3 and 7 days at 37 ^{o}C to evaluate the effects of water absorption on material properties. The pH and ions levels in PBS are similar to those in human saliva.

Acid Treatment

A portion of raw and thermoformed material samples were immersed in citric acid buffer (0.2 mol/L Na_2HPO_3 in 0.1mol/L $C_4H_2O_7\cdot H_2O$) of either pH of 3 or 5. The samples were soaked in the buffer for 1, 2, 3 and 7 days at 37 ^{o}C to evaluate the effects of low pH on material properties. The samples were wiped with Kimwipe before further analysis. Part of the samples that were soaked for 7 days were airdried in the fume hood under room temperature (21 ^{o}C) for 4 days to eliminate absorbed water.

III c. Developed Experimental Protocols

Crystallinity Characterization

DSC (Perkin Elmer, DSC 8000) analysis was used to characterize crystallinity of PETG, TPU-1 and TPU-2 materials. It can generate thermal graphs that allow us to identify the presence of crystal structures in aligners. Compared to DMA, it has several advantages. DSC testing can conduct at higher temperature range close to melting point, while DMA can only test around Tg. Second, there is no specific sample dimension requirement for DSC, while DMA requires rectangular sample shapes. Third, DSC testing can be conducted multiple times to obtain different information according to each heating/cooling curves, but DMA Tg testing can only test samples once as samples are deformed after each cycle. The heating rates for DSC were 20 ^{o}C/min for both heating and cooling processes. The testing was conducted twice, assuming that the first heating/cooling curves contained thermal history from heat treatment during thermoformation, and the thermal history was erased in the second heating/cooling curves. The samples cut from thermoplastic materials or clear aligners are round shape to fit well with the hermetic pans of DSC. The weight of samples was around 10 mg.

Glass Transition Temperature (Tg)

Tg was measured by two devices, DMA and DSC. DMA Q800 DMA provided more sensitive detection of glass transition compared to DSC. It can sense the transient stiffness differences between the glassy and rubbery states during phase transitioning. Samples from raw materials and aligners were cut (7.0mm x 2.2 mm) and loaded onto the DMA film tension clamps. DMA’s multi-frequency strain mode was used with the “temp ramp/freq sweep” test method at a frequency of 1.0 Hz. The samples were heated at a rate of 5^{o}C/min from 30 to 140^{o}C. Both raw materials (PETG, TPU-1) and aligners (PETG, TPU-1, TPU-2) were tested.

Microhardness

A digital microhardness tester (SE-1000DT) was used for microhardness testing. Vickers diamond indenter penetrator was pressed into the sample surfaces with a penetrator under 15-second automated load cycles. Vickers test the indenter is a diamond pyramid with a specific included angle. A constant load of 200 gf was applied to samples during each cycle, as it represent the average occlusal force level based on our calculations.[47] Three raw materials (PETG, TPU-1) were cut into 20 mm x 10 mm samples. Aligner samples (PETG, TPU-1 and TPU-2) were 5 mm x 5 mm in dimension. Microhardness was calculated based on the formula:

$$\text{Vickers hardness} = \frac{2\mathrm{F}\sin\left(\frac{\theta}{2}\right)}{\mathrm{d}^2}$$

where F is the load in kgf, θ is the angle of pyramidal angle of indenter, d is the arithmetic mean of the two diagonals of indenter).

Crack Resistance

Gardner^{TM} impact tester was used to measure the crack resistance. The impact tester measures the amount of energy absorbed before material fracture takes place.[25] Raw materials (PETG and TPU-1) with thicknesses of 0.76 mm were tested. The samples were subject to different impact forces created from dropping a standard object with a weight of 2 kg from different heights. 2 kg was selected as it resembles the occlusal impact force level reported by a previous study.[47] Indentations formed as a result of the impact forces. The indentation sizes were measured by a digital caliper. The energy absorbed from impact forces was calculated by:

$$E = mgh$$

where E is the energy absorbed from impact force, m and h represent the mass of the weight and height of the drop respectively.[25] An indentation vs. height plots were generated to compare the resistance of impact under different potential energies levels.

Light Transmittance

Light transmittance was measured by a spectrophotometer (Konica Minolta CM 2600D) for both raw or thermoformed TPU-1 and PETG materials. F2 visible Illuminant light with wavelength of 360-740 nm was used for light transmittance. The wavelength range was selected as it is within the vision detection range. Similar range was used in multiple previous studies. [5,19] The focus area of light was 8mm x 8mm. UV setting used was 0%.

Stress Relaxation

We developed protocols to generate consistent and reliable stress relaxation outcomes of aligner by using TA Instruments® DMA Q800. Raw materials (PETG and TPU-1) and aligner samples (PETG, TPU-1 and TPU-2) were cut into 7.0 mm x 2.2 mm strips. Aligner samples were obtained from the lingual side of the central incisor areas. The samples were loaded onto DMA tensile clamps. One batch of the samples was soaked in PBS and two others in Na_2HPO_3 acid buffers (0.2 M) with pH of 3 and 5 for specific time intervals of 1, 2, 3, and 7 days. A constant strain of 0.6% and preload of 0.5 N was applied to the samples at 37°C for 2 hours. 0.6% strain level was chosen because it represents the typical strain level that aligner materials are subjected to during clinical use based on our calculations. A preload was added to samples to account for the curvatures in the aligners that create inconsistencies in stress relaxation results. Unlike raw materials that are flat, aligners are curved as they follow the anatomy of tooth surfaces. The curvature must be reduced to accurately detect the actual stress relaxation produced from the 0.6%

strain exerted on the aligners. A preload is a force that was added on samples prior and during testing. It stretched the samples to became flatter so the added strain can fully express itself to produce consistent results. In our protocol, a preload of 0.5 N was found to produce the most accurate, reliable and consistent results. TA Analysis Software™ was used to analyze data and create stress relaxation plots. The parameters of tress relaxation were calculated as:

Stress relaxation= (Final stress-Initial stress)/Initial stress x 100%.

Stress relaxation velocity= (Final stress-Initial stress)/Testing hours.

Creep

We developed protocols to characterize the creep of different aligners by using TA Instruments® DMA Q800. Aligner samples of PETG, TPU-1 and TPU-2 were cut from the lingual of the central incisor (7.0mm x 2.2mm) and soaked in PBS for 24 hours and air dried in fume hood for 1 day. Samples were subject to tensile strength (0.6 MPa), which represents the ideal pressure level for orthodontic movement. The frequency sweep mode was used in DMA. The tested frequencies were 0.1, 0.32, 1.0, 3.2, 10.0, 31.6, and 50.0 Hz. Since creep is affected by changes in temperature, increments of temperature were applied to samples to generate graded deformation. The temperatures that the samples were subject to range from 34 to144 ^{o}C. A 10 ^{o}C temperature increment was applied to the sample over 160 minutes. For each temperature increment, a creep vs. temperature curve was generated by the DMA software. Ultimately all the curves that were produced from incremental temperatures were superimposed onto a reference curve generated at 34 ^{o}C. A time-temperature superimposition (TTS) master curve can be formed based on the superimposition by using TTS curve shifting software. From TTS curves, we were able to extrapolate predicted creep strain or compliance over weeks to even years.[44] In each master curve, there is a primary region that the creep rate decreases rapidly with time, a secondary region where the creep rate reaches a steady state, and the tertiary region that the creep rate increase again until rupture. Creep compliance was calculated as creep strain divided by applied load. Creep resistance was calculated by 1/creep compliance.

Arrhenius Energy

Arrhenius Energy was measured by DMA Q800. This system and the polymer viscosity obey the Williams-Landel-Ferry (WLF) equation:

$$\log A_t = \frac{-C_1(T - T_o)}{C_2 + (T - T_o)}$$

where T_o is reference temperature (K); T is the measurement temperature (K); C_1 and C_2 are constants; A_t is the shift factor which indicates the amount of shifting along the horizontal in TTS data to align individual experimental data points into a master curve.

Thus, Arrhenius Energy can be measured by DMA using frequency sweep mode. Aligner samples cut from the lingual of central incisor (7.0mm x 2.2mm) were soaked in PBS for 1 week and air dried for 1 day in the fume hood. Soaked and unsoaked samples were tested for tensile strength under 34-144°C at a rate of 10°C increment for 4 to 5 hours. The testing frequencies were 0.1, 0.32, 1.0, 3.2, 10.0, 31.6, 50.0 Hz, and the fixed strain was 0.6%.

Thickness Distributions

The thickness of thermoformed aligners of PETG, TPU-1 and modified TPU-2 were measured in the study. Both PETG and TPU-1 aligners were prepared from raw sheets with a thickness of 0.76 mm. TPU-2 aligners were used as received. Samples of all three aligners were cut from the central incisor, first premolar and first molar regions with a width of 1.0 mm. The thickness of the samples from the facial and lingual aspects were measured by a caliper. The thickness of each location was measure five times by the same examiner and the average thickness was calculated. The topographical distribution of thickness was mapped out.

Hydrophilicity

Hydrophilicity was measured by a drop shape analyzer (Kruss® DSA 100). The samples were placed in the sealed chamber of the analyzer and a liquid drop (0.5 μl) was placed on the sample surface at 37 ºC. The contact angles between the droplet and samples were captured by internal cameras. The wettability of the surface was calculated via Young's equation, based on initial and equilibrated sessile contact angles of the samples.

Water Absorption

The water absorption rates were measured by an analytical balance (XPE 504). Aligner materials (PETG, TPU-1, TPU-2) were cut (7mm x 2.2mm) and immersed in PBS at 37 ºC. At specific time points, samples were collected and weighed. The ratio was calculated as:

Water absorption ratio= $(W_t-W_0)/W_0 \times 100\%$,

where W_0 and W_t are initial and final weights respectively.

III Statistical Analysis

The Shapiro-Wilk test was applied prior to data analysis to assess the normality of each parameter. To compare the differences between the testing conditions and among different materials, data will be analyzed with the statistical software SPSS v17.0 (SPSS Inc., Chicago, IL, USA). Statistical tests were chosen based on appropriateness of the distribution and comparison to be made, and thus repeated-measures analysis of variance (ANOVA), student t test, multi-way

ANOVA, and Tukey's test will be used to determine the presence of statistically significant differences. 95% confidence interval and p values less than 0.05 are chosen by convention.

IV RESULTS AND DISCUSSION

IV 1. Aim 1

IV 1a. Crystallinity Characterization

The degree of crystallinity plays a determinant role in the mechanical properties of plastic polymers.[25] The aligner materials can vary significantly based on the amount of crystalized structures. Some aligner materials are amorphous, meaning there is little or no crystalized structures, while others can be more crystalized. The crystalized structure may confer higher thermal stability and provide more mechanical strength.[24] Therefore, it is crucial to understand the crystallinity of different materials in order to explain the different mechanical properties observed in different materials.

In our study, DSC was utilized to characterize crystallinity of the materials. The amorphous and crystalline plastics exhibit disparate thermal features that can be used to identify the degree of crystallinity of materials.[7,25,27] Figure 2 shows the thermal plots from DSC for PETG raw materials. No melting temperature (Tm) peaks were observed for PETG material samples, which suggests that PETG materials are amorphous plastics (Fig 2).

In contrast, the TPU-1 raw materials showed melting temperature (Tm) peaks at 165 °C and 194 °C. The melting temperature of TPU-1 spread over a wide range of from 150 °C to 200 °C (Fig 3). Occasionally, more than two or more melting peaks may be observed in TPU-1 samples. Collectively, the evidence suggests that the TPU-1 materials may have a semi-crystalline copolymer structure (AB type).

TPU-2 showed a recrystallization peak and a melting peak in the second heating curve. In addition, two crystallization peaks were observed in the cooling curves (Fig 4). These results suggested TPU-2 may be partially crystalline.

IV 1b. Glass Transitioning Temperature (T_g)

The mechanical properties of amorphous and crystalline plastics behave very differently around their glass transitioning temperatures (T_g). T_g evolution is considered the most used indicator revealing the degradation of the material structure.[34] Lijima et. al. stated that the orthodontic forces delivered by thermoplastic appliances may be influenced by the T_g of the materials.[8]

At T_g, the polymer chains acquire sufficient energy and the matrix structures become mobilized, resulting in a change of physical states. Plastics solidify to a glassy state at temperatures lower than the T_g, but quickly soften to a rubbery state when temperature exceeds Tg.[25] At T_g, crystalline plastics remain intact and firm. The amorphous regions within the plastic have weak intermolecular forces and they can be mobilized at this temperature. In contrast, as amorphous plastics have no crystalline regions, they become soft and flexible elastomers or highly viscous liquids above Tg.[24] Thus, amorphous and crystalline polymers vary drastically in their mechanical properties at T_g. Aligners are subject to temperature changes during fabrication and intraoral usage. The characterization of Tg will help us to predict mechanical property changes during thermal aging processes.[8,17,27,32]

In our study, DMA was used to characterize Tg of different materials. TPU-1 raw materials showed a higher Tg (80.29 ± 2.73 ^{o}C) than PETG raw materials (68.60 ± 2.19 ^{o}C) (Fig 5; Table II). These results are consistent with our data on the degree of crystallinity of the two materials.

Crystalline plastics have crystalized structures that confers higher thermostability, resulting in a higher T_g.[25] On the other hand, amorphous plastics do not have crystal structures. Instead, they have loosely organized bundle-like structures whose orientation can be easily altered by increase in temperature. Therefore, amorphous plastics tend to have lower T_g than crystalline plastics.[34] Since TPU-1 is a semi-crystal plastic while PETG is an amorphous plastic, the T_g if TPU-1 is higher than that of PETG.

The T_g of the two materials are found to be much higher than intraoral temperature (37 ^{o}C). These results are consistent with the findings in other studies on polyurethane and PETG based materials.[24,34,45] The high T_g of these materials can be attributed to the benzene rings groups in their structures.[16]

Based on the T_g values, we can predict that at room (21^o) or intraoral temperatures (37 ^{o}C) the mechanical properties of these two aligners will not be likely to change as these temperatures are well below the Tg of these materials. A previous study reported that oral temperatures can increase to 57°C when ingesting hot liquids.[46] The T_g of the materials are above this threshold, and thus, it can be assumed that even after consuming a hot beverage, these materials would not change significantly in their properties. However, if these materials are exposed to temperatures above T_g, such as if the aligners are placed under direct sunlight for a prolonged period of time, physical and chemical compositions of these materials can be impaired, leading to potentially compromised clinical results.

IV 1c. Microhardness

Microhardness measures a material's resistance to penetration on a microscopic scale.[25,33] During clinical use of aligners, the materials are subject to large occlusal forces from chewing and clenching. During maximum intercuspation, the forces withstood by occlusal contacting areas of molars can range from 107 to 156 Newton.[47] Such high level of forces is sufficient to cause localized indentations in the aligner surfaces.[33] Schuster et al. reported that microhardness of aligners was found to be greatly reduced after clinical use.[42] The observed difference might be accounted by change of the polymer crystallinity resulted from masticatory loads induced cold work.[42] The resulted deformation of aligners can be rather undesirable, as it can cause misfit of aligners over time;[30] and change the directions and intensities of forces generated to teeth or generate unwanted orthodontic movements. Therefore, aligner materials that have high microhardness are more favorable.[25]

In our study, we developed protocols to characterize microhardness of the raw materials and thermoformed aligners. During the testing, the materials were indented until an impression was formed. The sizes and surface microstructures of the resulted indentations were measured with a calibrated optical microscope. The structures and dimensions of the created indentations reflect the hardness of a material to counteract deformation from external forces.

For the raw materials, TPU-1 had higher microhardness than PETG (Fig 6). We did not include raw modified TPU-2 as they were not available to the general public. The results indicate that TPU-1 is more likely to withstand the large occlusal forces and resist penetration or indentation caused by occlusal forces than PETG.

IV 1d. Crack Resistance

Crack resistance is the ability of a material to absorb mechanical energy in the process of deformation and fracture under impact loading.[25] In other words, this property reflects how much force can be applied to a material before it starts to fracture and break.[20] When the exerted force exceeds recovery point of a material, failure of the bonds between the polymer chains begin to accelerate and microscopic or macroscopic cracks will ultimately take place.[7]

Clinically, materials with high crack resistance are superior. It is found that after 2-week of clinical wear, cracks, voids and delamination were found on the surfaces of aligners.[13] Repeated insertion, high occlusal forces during chewing or bruxism can lead to the appearance of abrasions and cracks in aligner materials.[2,13] Cracks and delamination can significant weaken the strength generated by clear aligners, rending treatment ineffective.[18] In addition, it is reported that the cracks may contribute to the leakage of elements of aluminum, nickel and zinc from certain aligners, which may pose a danger to people who are allergic to these elements.[13]

In our study, we developed protocols to analyze the crack resistance of both raw and thermoformed aligner materials. The concepts behind the testing protocols of crack resistance are similar to those of microhardness. A weight was dropped from a measured height and the impact energy needed to cause initiation of crack was determined. The higher the weight is lifted, the higher the impact energy transferred to the material under test. The smaller the resulted indentation size, the more crack resistant the material was deemed.

The TPU-1 raw materials cracked at significantly higher height (i.e., impact energy) than PETG raw materials (Fig 7). Thus, TPU-1 materials absorbed significantly more energy than PETG before fracturing, indicating a higher crack resistance of TPU-1 raw materials.

IV 1e. Light Transmission

Light transmission an optical property that reflects the transparency of clear aligners.[19] A higher light transmission is desirable as it provides more aesthetic appearance during clinical wear.[5] It is reported that there is a wide range of light transmission of aligners.[19] Transparency of aligners depends on the manufacturing processes, as well as chemical compositions and physical structures of different aligner products.[5,18,19]

The PETG raw materials showed a greater transparency than TPU-1 (Fig 8). The difference may be attributed to the chemical compositions of the two materials. It is reported that the degree of crystallinity of play an important role in light transmittance of materials.[5] Higher crystallization contents may contribute to the reduced transparency and increased opaqueness of a material.[2,5,18,19] The structures of PETG materials were mostly amorphous while those of TPU-1 materials were semi-crystalline. Amorphous structures allow light to pass through and thus increase transparency of a material. On the other hand, in semi-crystalline polymers, the bundle-like crystalline regions scatter visible light and increase material opaqueness.[25,48] Therefore, our results are consistent with the findings in crystallization characterization.

IV 1f. Stress Relaxation

Stress relaxation is a unique property of thermoplastic polymers. It describes the phenomenon that amount of force or stress exerted by a material that gradually decreases in response to a constant deflection.[25] In orthodontic treatment, the forces delivered by aligners decay with use.[13,26] The higher the stress relaxation, the more force is “relaxed” or lost over time and thus less force is delivered to teeth for orthodontic movement.[13,26] Different aligner materials have various stress relaxation rates depending on their chemical and physical compositions.[13,16,37] Lombardo et al. investigated four thermoplastic plastic sheets (PETG, TPU, copolyester/PETG, TPU/PETG) and reported that the 24-hour stress relaxation of these materials varied significantly, ranging from 15-44%.[16] Fang et al. studied five different raw aligner materials made from PET, PETG and coplyester in simulated oral conditions (37°C in water bath). The authors found that only around

42-66% of initial forces generated by the materials were delivered after 3 hours of testing.[26] Elkholy et al. investigated the thermoformed aligner materials made from different standardized stone blocks with the different geometries. The results indicated that 44% to 76% of initial force was lost in these materials after 24 hours of testing.[37] However, these study results have limited clinical applicability since none of them tested aligners used in clinical settings. The previous studies measured either raw material sheets or thermoformed aligners made from stone models rather than actual teeth. As the complex geometry of teeth can greatly modify the thickness and mechanical properties of the materials, [21,24,37,40] it is crucial to examine and quantify the stress relaxation of actual aligners to determine the remaining force being delivered for tooth movement.

In our study, DMA was used to quantify stress relaxation of raw materials (PETG and TPU-1) and thermoformed aligners (PETG, TPU-1 and TPU-2) (Figure 9). For raw materials, TPU-1 delivered larger initial stress compared to PETG (Fig 10). However, the stress relaxation rate of TPU-1 (51.2%) was similar to PETG (49.74%) (p=0.53) (Fig 10, A; Table III). After 2 hours of testing, the remaining stress generated by both raw materials were comparable (p=0.37) (Table III). The velocity of stress relaxation of TPU-1 (2.17 ±0.31 MPa/hr) was greater than that of PETG (1.94 ±0.22 MPa/hr), which means TPU-1 lost its forces at a higher rate (Fig 10, B; Table III). The remaining orthodontic force levels after relaxation were similar for both materials. Only about 50% of original force level was generated in both PETG and TPU-1 raw materials after 2 hours.

IV 1g. Creep

Creep describes the tendency of aligner materials to deform permanently under constant forces below the yield point. The deformation can take place over slowly a long period of time.[25] Creep can be used to describe the deformation that is accumulated gradually under constant orthodontic loads or intraoral forces.

Some small degree of creep may be arguably beneficial. Small space between an aligner and teeth resulted from creep may act as a buffer to mitigate excessive forces delivered to PDL of teeth during retention phase. Thus, small amount of creep may promote patient comfort during tray insertion or chewing without significantly affecting clinical outcomes.[15] However, if excessive creep takes place before desired orthodontic movements are achieved, the aligners will not adapt well to tooth surfaces. Patients may experience pain or discomfort when they wear the maladapted trays so they may be unwilling to cooperate with the treatment. As aligners are meant to be worn 22-24 hours a day to achieve desired outcomes,[1] reduce patient compliance may greatly compromise clinical results. In addition, even if patients were complaint, the distorted trays can alter the magnitude of forces or change the moment vs forces ratios for orthodontic movements. The clinical results will be greatly compromised.

Creep may also undergo time-dependent changes like stress relaxation.[13] Bradley et al. found that aligner materials became more brittle and less resistant to creep with increased clinical use.[49] Therefore, it is important to quantify the creep of aligners and elucidate the how creep changes with time.

In our study, we developed a protocol to characterize creep with DMA. TTS technology was used to create a mathematical model that predicts creep changes over weeks to months based on data obtained from 2-3 hours of testing in DMA (Fig 11). From TTS, we calculated creep compliance and modulus, which showed how easily the aligners can deform with use.

The DMA TTS data suggest that PETG had more creep compliance and less creep modulus than TPU-1 from week 0 to week 4 (Fig 12, A and B; Table IV and V). Creep compliance indicates the ease of a material to deform. The higher the compliance, the easier a material can deform over time. Creep modulus was calculated as 1/creep compliance, which indicate the resistance of a material to creep. The results indicate that TPU-1 was less susceptible to creep over time compared to PETG. PETG raw materials are more prone to deformation during clinical use.

In addition, the data show both PETG and TPU-1 materials underwent time dependent changes in creep. The creep compliance of both materials increased significantly from week 0 to week 2 (Fig 12, A). The most increment of compliance was observed from week 0 to week 1. From week 1 to week 2, compliance continued to increase but at a lesser rate. From week 2 to week 4, there was no significant difference of compliance for both materials (Table IV and V). The results of modulus are consistent with the findings in compliance.

In summary, TPU-1 was much more resistant to creep than PETG at all time points. Both materials increased creep by 3-fold over time. However, most of the changes in creep occur during first week of use. The change continues till week 2 then plateau afterwards. As most aligners are worn for 1 to 2 weeks clinically, [1] the dramatic reduction in creep may compromise clinical outcomes. TPU-1 was superior to PETG in terms of creep resistance.

IV 1h. Arrhenius Energy:

Arrhenius Energy (E_a) is a fundamental parameter routinely measured in thermo-aging tests for plastics. It plays an important role in evaluating aligners as it indicates the minimum energy required to allow transition between phases in polymers.[25] Arrhenius energy can be used to corroborate our findings on mechanical properties from an energetics perspective. For example, it can be used to provide a quantitative description of creep deformation increase exponentially with increase in temperature. In Arrhenius equation, the deformation rates are reversely related to the amount of Arrhenius activation energy. Besides from creep, it is also used to model the temperature variation of diffusion coefficients, population of crystal vacancies and many other thermally induced reactions. If a material has a high E_a, it will require more external thermal or mechanical energy to destruct the molecular interactions and chemical bonds to initiate a change in its structure.[25] Materials with higher activation are more resistant to time-dependent degradation of thermomechanical properteis.[25,27]

In our study, we developed protocols to quantify E_a by DMA. The Ea of TPU-1 raw material (418 ± 3.12 KJ/mol) was significantly higher than that of PETG raw material (403 ± 3.77 KJ/mol) (p=0.04) (Table VI).

The E_a results are consistent with our previous findings on the mechanical property differences of the two raw materials. PETG was found to be an amorphous plastic with a lower Tg while TPU-1 was a semi-crystalline material with higher Tg. It requires more energy to induce phase change in crystalline polymers as crystal structures consume more energy than the amorphous structures.[25] Therefore, the activation energy is usually higher in crystalline polymers such as TPU-1. The E_a results can also explain why PETG had lower crack resistance, microhardness. Because PETG had smaller Ea value, it may be more susceptible to the structural degradation from external impact. Therefore, PETG were found to be more prone to deform, crack and break with time.

IV 2. Aim 2

During fabrication, the raw materials undergo heat treatment to form clear aligners. The raw thermoplastic sheets are heated to a suitable temperature according to material manufacturers recommendations with a heating unit. The materials are heated till they sag slightly. Then they are dropped quickly onto a dental cast under vacuum suction device to ensure softened materials can firmly adapt to dental cast surfaces. The thermoforming processes can precisely replicate the anatomy of patient models.[7,24,50]

However, it is reported that the thermoforming processes may significantly alter the physical and thermal properties of original plastic materials. The properties such as thickness, [7,17,19] transparency, [5,18,19,50] [5,18] glass transition temperature,[8,32,37,51] physical structures,[42] elastic modulus, [13,15] deformation, [17] water absorption[14,19,24,37,43,52] and forces exerted by the materials.[28,29,40] can be impaired during heat treatment. The most obvious change is that the thickness of obtained aligners is much smaller than their respective raw materials.[24] An abundant number of previous researches characterized the property changes of either raw materials sheets or thermoformed materials fabricated from stone models rather than actual teeth. However, the property changes resulted from heat treatment on actual aligners used in clinical settings need to be elucidated.

IV 2a. Effects of Heat Treatment on Crystallinity

It is well established that the change in temperature can modify the degree of crystallinity of plastic materials.[8,25,34] When raw thermoplastic sheets are heated above glass transitioning temperature Tg, the crystal bundles in crystalline plastic can undergo structural disassociation. When materials start cooling down, crystal structures can begin to recrystalize.[25] Depending on the thermoprocessing techniques, the change in the amount of crystal structures in crystalline polymers can vary. It is reported that in general crystalline polymers show an increase of crystallinity after heat treatment, and thus the tensile strength and elastic modules would be enhanced accordingly.[38] However, Ryokawa et al. investigated eight thermoplastic materials with different compositions (Polyurethane, polypropylene/ethylene, copolyester, polycarbonate, polyethylene, PETG, ethylene–vinyl acetate) in simulated oral environment, and found that the degree of crystallinity decreased in almost all of the materials after thermoformation. The author attributed the results to the rapid cooling process in aligner fabrication that crippled the recrystallization process.[24] However, the thermoformed samples tested in this study were not aligners used in clinical settings. Therefore, there is a need to quantify the change of crystallinity from raw materials to aligners used clinically.

Our DSC results showed that the no melting temperature peaks were observed in both PETG raw materials (Fig 13, A) and thermoformed clear aligners (Fig 13, B). The lack of melting temperature peaks indicates that PETG materials were amorphous plastics. The glass transitioning peaks on the very top of heating curves of both raw and aligners is an indicator of structural phase change resulted from heat treatment. The peaks for both raw materials and aligners were similar (around 82 ^{o}C) (p=0.44). The data suggest that there was no thermal history of phase change from the heat treatment in PETG, and we can infer that the microstructure of the PETG remained unchanged.

On the other hand, the heat treatment process had significant influence on the crystallinity of TPU-1 materials. For the first glass transitioning peaks. A melting temperature peak was observed in both TPU-1 raw and aligner materials (Figure 14, A and B). The presence of melting temperature peaks signals for existence of crystal structures. The overall thermal patterns of the curves suggest that both raw and aligners of TPU-1 were semi-crystalline. The glass transitioning peaks on the very top of heating curves of both raw and aligners indicate of structural phase change occurred from heat treatment. The thermal peak of raw material (79 ^{o}C) was shifted to higher temperature (83 ^{o}C) in aligner material. The corresponded enthalpy change was increased from 0.75 to 1.2 J/g after heat treatment process. The increase in enthalpy suggests that the materials structures became more disordered. In other words, the data suggest that there was an increase of amorphous phase in TPU-1 after heat treatment. According to the enthalpy energy calculated from the melting temperature peaks, the enthalpy was decreased from 2.0 J/g in raw material to 0.6 J/g in aligners. This data indicate that there was a partial breakup of crystalline structures during heat treatment. In conclusion, TPU-1 demonstrated a shift from semi-crystalline to amorphous structures after thermo-processing. It is possible that the heating processes caused tensile elongation of polymeric chains that subsequently led to break down the crystalized structure. [25] However, during cooling period, the rapid drop of temperature prevented the crystal structures to fully recovery to its original degree.[24] Therefore, the overall result was a reduction of crystallinity of TPU-1.

IV 2b. Effects of Heat Treatment on Glass Transition Temperature (T_g)

Tg can be modified by heat treatment during aligner fabrication or thermal aging in clinical use.[34,45] The changes T_g before and after heat treatment can have a direct implication on the changes of mechanical properties from thermoforming processes.[8,27,32,34,51] During heat treatment, the thermoplastic raw plastic sheets are heated to a temperature that is above $T_{g.}$ In this process, the materials absorb heat which translated into energy that mobilizes polymeric chains and breaks molecular interactions within the plastics. If the materials were slowly cooled down to a temperature below T_g, they may revert back to their original configurations.[25] However, the cooling process in aligner thermoformation occurs much faster so full structural reversal may be prevented.[34] The mobilized structures reassociate and molecular bonds are formed haphazardly from the rapid cooling processes, resulting in a different composition of material compared to the raw materials. The changes in structures and compositions alters the mechanical properties of aligners. The change of Tg in response to thermal challenges various between products, depending on their original material types.[25] Previous studies studied the effect of heat treatment by comparing original materials and thermoformed material made from stone models.

In our study, we quantified the T_g of aligners made from impressing on patient dental models and compared with that of raw materials (Fig 15). The Tg of PETG raw materials was 68.60 ± 1.21°C while that of aligner was 67.46s ± 0.21°C (Table VII). The Tg of PETG did not change significantly after heat treatment (p=0.33). Base on the results, it can be concluded that heat treatment did not modify the microstructure of PETG and the mechanical properties of PETG aligner would be consistent with the raw material.

TPU-1 materials, on the other hand, was considerably influenced by heat treatment process (Fig 15, Table VII). The Tg of PETG raw material was reduced significantly from 80.29 ± 1.31 °C to 75.70 ± 0.68 °C after heat treatment. The reduction of Tg indicates that the molecular structures of TPU-1 was altered, and phase transition occurred at a significantly lower temperature after heat treatment. Consequently, the mechanical properties of TPU-1 such as stiffness may be reduced after thermoformation.[24] This result is consistent with the characterized crystallinity changes in TPU-1. Plastics with higher amount of crystallinity tend to have higher Tg as crystal structures require more energy to undergo phase change.[25] Thermo-processing led to breakdown of crystalized structures in TPU-1 and thus the Tg was reduced.

Despite the reduction of Tg in TPU-1 after heat treatment, the Tg of TPU-1 aligners (75.70 ± 0.68 °C) was still significantly higher than that of PETG aligners (67.46 ± 0.23°C). However, both of these aligners had much lower Tg compared to TPU-2 aligners (118.2±1.76) (Table VII). Since the Tg of all three materials are much higher than the room temperature (25°C), normal intraoral temperature (37°C) or intraoral temperature after consuming hot beverages (57°C)[46] , their mechanical properties are not likely to be significantly modified in these environments. However, TPU-2 aligners are significantly more resistant to mechanical changes under more extreme thermal conditions.

IV 2c. Effects of Heat Treatment of Aligner Thickness

It has been reported by multiples studies that the thickness of thermoplastic materials is reduced significantly after heat treatment.[7,19,24]. Ryokawa et al. reported that the thicknesses of eight thermoplastic products were reduced to 74.9 to 92.6% of original dimensions after thermoformation.[24] The thickness of aligner materials is related to the mechanical performances of aligners, such as elastic modulus[19,24], yield stress [24], tensile force[7], water absorption[7,19], transparency[19], surface hardness[19], deflection[7,17,21], crack resistance[7]. Therefore, proper thickness of thermoplastic materials needs to be determined to achieve optimal mechanical properties for orthodontic tooth movement.[17]

Within the same aligner, the thickness is not evenly distributed. However, none of the existing literatures studied the topographical distribution of aligners used in real world. Most of studies measured thickness of materials that were thermoformed onto a standard object such as a stone model rather than teeth[7,8,19,24]. The complex geometrical form of teeth may significantly alter the thickness distribution and local mechanical properties of materials.[24] Thus, it is important to characterize thickness of actual aligner to draw clinical implications.

In our study, we mapped out the thickness distributions of three aligners made from the same set of patient dental models (Fig 16). The TPU-2 aligners were measured as received from the aligner company. The thickness of raw materials of PETG and TPU-1 were 0.76 mm. TPU-2 raw materials are proprietary to the manufacturer and not available to the public. However, Ryokawa et al. reported the raw materials for TPU-2 aligners was 0.76 mm,[24] which is consistent with the thickness of the PETG and TPU-1 raw materials (Table VIII). Therefore, the thickness of the three raw materials is the same.

The reduction of thickness of the three plastics after heat treatment was significant. The thickness distributions of the three aligners were measured (Fig 17-19). Only 50-70% of the original thickness of PETG and TPU-1 was measured on fabricated aligners (Table IX and X). The facial thickness of the central incisor of TPU-1, PETG and TPU-2 aligners decreased from incisal to gingival portions, potentially from premature cooling at the incisal edge and stretching of the materials gingivally. However, the thickness of the lingual aspect of all three materials was similar from incisogingivally, possibly from decreased stretching forces due to the anatomy of cingulum (Fig 17-19). For premolar and molar samples in all three materials, the thickness was greatest at the occlusal portion, then gradually reduced gingivally. However, the thickness of buccal and lingual sides for premolar and molars were similar (Table X), potentially due to the symmetric morphology of these teeth. For PETG and TPU-1 aligners, the thickness of the lingual portions of incisors was significantly greater than the facial, but the TPU-2 aligner had similar thicknesses in both facial and lingual portions ($p=0.22$). In addition, TPU-2 aligners had larger thicknesses in molar and premolar regions compared to PETG and TPU-1 aligners. These results showed the aligners were much thinner than their original materials, and the thicknesses were not evenly distributed within the same aligner.

The thickness changes from heat treatment may be attributed to by multiple factors. The difference between the heating temperature and the Tg plastics, heating duration, and molecular weight of polymer chains, composition of functional groups are all related to the extent and speed of shrinkage.[24] During thermo-processing, the original raw sheets are heated to a temperature which is well above the Tg measured for the three materials. As shown previously, TPU-2 had the highest Tg of all three materials. The higher Tg to heating temperature difference may explain why TPU-2 materials had the smallest thickness reduction and most even thickness distribution compared to the other two materials. The Tg of TPU-1 was higher than that of PETG materials. However, TPU-1 had the greatest reduction of thickness of all three materials. This is likely resulted from the breakdown of crystalline structure of TPU during heat treatment while PETG remain amorphous and thus was not affected by heat as much. It is also possible that heat treatment reduced the average molecular weight of TPU-1, which made the plastic prone to shrinkage during thermoformation. This is supported by another study where the authors investigated another type of TPU and found the molecular weight loss caused more reduction of thickness.[24]

The clinical performance of clear aligners can be profoundly influenced by the thickness of materials. Mechanical properties such as elastic modulus, [24] deflection level, [7] light transmission [50] and force delivered [17,29] are directly related to the thickness of the aligner materials. However, our data showed that the thickness of aligner was not evenly distributed. The longer the distance the raw materials were stretched from its original position, the thinner the materials became. Thus The thickness was generally found to be thicker in incisal or occlusal regions than the cervical regions. As the force generated by aligners is directly proportional to its thickness,[17,28,29,40] the uneven thickness distribution may alter the designated tooth movement.

For example, if a buccal crown torque is planned in treatment to flare upper incisors, it would require more force at the cervical region of crown than the incisal region. The force needs to be closer to the center of resistance of the tooth in bone to generate a moment that would procline the incisors. However, if more force is generated at incisal region, a lingual crown torque would result. Our data showed the cervical regions were thinner than the incisal portion. Thus, the buccal crown torque may be hard to achieve as more force is generated at the incisal region. This can explain why in clinical setting it is more difficult to use aligners to treat class II division II cases which require proclination of incisors.

IV 2d. Effects of Heat Treatment on Hydrophilicity

Hydrophilicity is used to quantify a material's proclivity to attract water molecules.[25,53] Hydrophilicity is measured by the contact angle between a liquid droplet and a material surface. Contact angle is a convenient way to assess the wettability properties of material surface and provides information on the interaction energy between the surface and the liquid. [54-56]

Traditionally, it may be difficult to quantify hydrophilicity of aligners with most popular testing methods as a flat sample surface is required. However, aligner surfaces are usually curved. In our study, Kruss® DSA 100 was used to take accurate hydrophilicity measurements on curved surfaces of aligners based on equilibrium contact angles (Fig 20).

The contact angle of thermoformed TPU-1 was 61.20 ± 1.3°, which was significantly smaller than that of thermoformed PETG (76.67 ± 0.38 °), and TPU-2 (70.01 ± 2.39 °) (Fig 21, Table XI). A lower contact angle indicates increased hydrophilicity. Thus, TPU-1 was more hydrophilic than TPU-2 or PETG. Interestingly however, raw PETG (88.01±0.44 °) and TPU-1 (76.89±0.31°) materials exhibited higher contact angles than heat-treated samples ($p=0.02$), suggesting thermoforming processes can increase the hydrophilicity of both materials. Our results are in good accordance with the findings from of Ryu et al.'s the study, in which they found the water absorption rate of four aligner materials (PETG and copolyester based) all increased after heat treatment. The hydrophilic tendencies are largely dependent on the chemical bonds present on a material surface. [5,18] Polyurethane polymers such as TPU contain the polar surface groups such as '–NHCOO–'. These polar groups can form hydrogen bonds which facilitate water adsorption into the material. In contrast, PETG polymers mostly contain surface groups such as '-COO-' and 'C-O-C', which are less polar than '–NHCOO–'. [57] As a result, PETG materials are more hydrophobic. During heat treatment, it is possible that the molecular structures relax and rearrange so that the polar groups are exposed to the surface of polymers.[56] Thus, materials have increased abilities to attract water after thermodynamic changes.

In the future, FT-IR, XPS and NIR spectroscopic studies need to be performed to characterize the molecular and macroscopic changes of aligner materials to elucidate the surface changes after heat treatment.

IV 2e. Effects of Heat Treatment on Water Absorption Ratios

During clinical use, water molecules attach to the material surface and are subsequently absorbed into the material.[24] The process of water absorption can facilitate the adsorption and attachment of the pigments and bacteria to the material surfaces. [2,5]

The water absorption of raw materials of TPU and TPU-1 were 0.34 % and 0.67% (weight %) respectively after 1 week of immersion in PBS. (Table XII). This result is consistent with our previous data on hydrophilicity, where TPU had higher hydrophilicity than PETG.

After thermoformation, the water absorption rates of TPU-1 and PETG were altered (Fig 22). At one week of PBS immersion, TPU-1 aligner had significantly more weight increase (0.91%) compared to that of its raw material (0.67%) (p=0.02). PETG also showed slightly higher absorption rate (0.44%) compared to its raw material (0.34%), although the difference is not statistically significant (p=0.26). The water absorption of TPU-2 was similar to that of PETG. The water absorption of TPU-1 aligner (0.91%) was about twice of that of PETG and TPU-2 (0.45%) (Table XIII). Thus, heat treatment significantly increased the water absorption of TPU-1 compared to PETG. TPU-1 may be more susceptible to the changes in mechanical properties due to its higher water absorption capacity compare to PETG.

In addition, the amount of volumetric swelling resulted from water absorption was also modified after heat treatment. Before heat treatment, the thickness of TPU-1 and PETG raw material increased by 7.9% and 5.3% respectively (Table XIV). The difference was not statistically significant (p=0.31). After thermoformation, TPU-1 aligners demonstrated the highest change in hydroscopic expansion, as the thickness increased by 14.3% more. PETG and TPU-2 had 10.7% and 7.0% increase in their dimensions (Table XV). The hydroscopic expansion was significantly increased for both PETG and TPU-1 materials. Therefore, heat treatment induced material swelling in both PETG and TPU-1 materials.

Previous studies have found that the degree of crystallinity of materials can influence the amount of water absorption and hydroscopic expansion. Amorphous plastics generally have higher water absorption rates than crystalline plastics, as the absorption of water is dependent on the free volume within macromolecular structures. Amorphous plastics have more free volume compared to crystalline plastics because they do not have crystalline structures. Consequently, amorphous material can absorb more water.[19,25,27,34,58] TPU-1 underwent breakdown of crystal structures during heat treatment, and its structures shifted from semi-crystalline to amorphous. The reduction in crystal densities provided more vacancy in its structures and thus we observed more dimensional expansion and water absorption compared to crystalline plastics such as TPU-2. The increase in PETG water absorption after heat treatment may be explained by decrease in molecular weight during heat treatment. Our results are consistent with previous studies by Ryu et al.[19] and Boubakkri et al.[34,45] where they found that thermoformation increased the water absorption of both amorphous and crystalline materials.

In addition to the difference in crystallinity, the chemical compositions of the materials may also contribute to the observed differences in water absorption. Ryokawa et. al also found that polyurethane (PU) materials exhibited greater water absorption rates than polycarbonic (PC) and PETG materials in a simulated intraoral environment.[2,24] Multiple studies reported that the polyurethane materials contained more polar functional groups than PETG polymers.[10, 19,57] Our data was consistent with the previous findings. Interestingly, the water absorption of TPU-2 group was more similar to PETG than TPU-1. Studies show that chemical compositions (ester or ether-based PU), addition of different plasticizers during manufacturing and thermoprocessing conditions can lead to the difference in water storage in different PU products. [14,15,48]

IV 2f. Effects of Heat Treatment on Stress Relaxation

It is reported that the temperature change in heating processes can alter the rate of stress relaxation in the materials. [8] In our study, DMA was used to quantify the stress relaxation changes after heat treatment for TPU-1 and PETG materials (Fig 23-24) . After heat treatment, the initial stress of TPU-1 reduced from 8.66 ±0.51 MPa to 7.85 ± 0.62 MPa, while the change in PETG was insignificant (p=0.63). (Table XVI-XVII); Before heat treatment, the 2-hour stress relaxation of PETG and TPU-1 were 49.74% ±3.21% and 51.20% ±2.03% respectively (Table XVI). After thermoformation, the rate of relaxation of PETG and TPU-1 both increased to 72.89% ±3.01% and 78.52% ±3.41% respectively (Table XVII). Both materials demonstrated significant increased velocity of stress relaxation after heat treatment. The velocity of stress relaxation increased by around 28% from 1.94 ±0.22 MPa/hour to 2.72 ±0.34 MPa/hour in PETG, and 31% from 2.17 ±0.31 MPa/hour to 3.13 ±0.36 MPa/hour in TPU-1 (Table XVI-XVII).

Our findings are in good accordance with previous studies. Ryokawa et al. reported that heat treatment reduced tensile yield stress and increased elastic modulus of both amorphous (PETG, PC, copolyester) and crystalline plastics (PP, PE, EVA). The author attributed the reduction of mechanical properties to the decreased molecular weight resulted from heat treatment of the plastic materials.[24] As the heating temperature generally exceed Tg of the tested materials, [2,34,45,56] the drastic temperature increase may induce strain in the polymeric structures and eventually result in fragmentation of molecules and thus reducing the average molecular weight of the treated materials. [32,59] Plastics that had addition of a stabilizer that could control the decrease in molecular weight during heating, had better mechanical performances.[24] In our study, we found that heat treatment reduced initial stress and increased stress relaxation amount and velocity of both semi-cyrstaline TPU-1 and amorphous PETG materials. Thus, it is likely that heat treatment process reduced the molecular weight in these materials, leading to the compromised mechanical performances of the two materials.

The change of crystallinity from heat treatment can also contribute to change in stress relaxation. As mentioned in section IV a & b, the crystalline structures of TPU-1 were fragmented during the heating process. The recrystallization processes were inhibited by rapid cooling cycles. The resulted structure after cooling was weaker than that of the original material. On the other hand, the heating process did not affect PETG as much, since it was amorphous and thus there was no change in crystallinity after heat treatment. Therefore, heat had more impairment on the mechanical properties of TPU-1.

Furthermore, previous studies have also pointed out that thickness of materials play an important role in the amount of initial tensile force [17,19,21] and stress relaxation [7,19,37, 32] of aligner materials. Therefore, the reduced thickness of both PETG and TPU-1 materials from heat treatment also partially contributed to the increase in stress relaxation.

When we compare the stress relaxation of the three thermoformed aligners, TPU-2 aligners had smallest initial stress and demonstrated the largest stress relaxation among all three tested thermoformed aligners (Fig 25). TPU-1 aligners had highest initial stress. The stress relaxation of TPU-1 was significantly higher than that of PETG. The initial stress levels of PETG and TPU-2 were not statistically different. The remaining stress after 2 hours testing was highest in PETG (2.03 MPa), followed by TPU-1 (1.69 MPa) and TPU-2 (0.96 MPa) (Table XVIII). These results showed that TPU-2 generated smallest force and lost most force during relaxation. As a result, the final force generated by TPU-2 was only about half of that of PETG. We chose the lingual of central incisors of three aligners because they all have similar thickness, and thus the stress relaxation results are irrelevant of the thickness. (Table XIX).

In conclusion, heat treatment reduced the initial force that the plastics can deliver during initial clinical insertion onto teeth. In addition, the process also increased stress relaxation of the materials, meaning that more force is lost with time as patient wear the trays for longer period of time, and thus compromising the clinical outcomes. The degradation of mechanical performances is likely due to reduction of molecular weight, crystallinity and thickness of the materials after heat treatment.

IV 2g. Effects of Heat Treatment on Creep

After heat treatment, the creep compliance of both thermoformed PETG and TPU-1 increased significantly compared to their respective raw materials (TPU-1: p=0.001; PETG: p=0.003) (Table XX). As a result, the creep modulus of both materials decreased. The results suggested the aligner materials were more prone to undergo deformation after heat treatment. The decreased creep can be attributed to two factors. First, the thickness of both materials decreased significantly after thermoformation. Studies have shown that reduced thickness can lead to significant increase in the tendency of materials to creep.[7,15,17,22] Second, since the heating temperature was above Tg of both materials, the high temperature can cause structural fragmentation and molecular weight reduction,[24] which can cause the materials to be less resistant to creep.[8]

Interestingly, heat treatment has more effect in TPU-1 than PETG materials. The creep modulus of TPU-1 reduced to 14.2% of its value before heat treatment, while that of PETG reduced to 23.3% of its original level (Table XXI). The difference of the reduction of the two materials is significant (p=0.03). The results indicate that TPU-1 was more susceptible to the effect of heat treatment as TPU-1 the reduction of creep modulus was larger than that of PETG. The greater change in TPU-1 can be corroborated with our data on DSC structural characterization and Tg. Tg of TPU-1 declined after heat treatment while that of PETG remained the same. The change in Tg indicate a reduction of crystallinity of TPU-1 after heating. The breakdown of crystal structures may lead to the degradation of mechanical properties including creep.

Among all three thermoformed aligners, TPU-1 showed a significantly higher creep modulus than PETG and TPU-2 within the predicted 4-week period (Table XXII and XXIII), which indicates that TPU-1 aligners are least prone to deformation during clinical use. TPU-2 had the biggest creep compliance, suggesting a susceptibility to distort over time (Table XXIII However, it is worth mentioning that the creep would likely be modified during intraoral use. Heat treatment led to structural disorganization which increase entropy of the system.[25] The process is accompanied by release of energy. The results of structural impairment may contribute to increase creep in thermoformed aligners. During intraoral use, the constant occlusal function such as occlusal contact, chewing and clenching may exert force on aligner material, and thus replenishing the lost energy within the system. Therefore, the actual creep may be less than our data suggest. Future in vivo study can be performed to confirm the change of creep during intraoral use.

IV 2h. Effects of Heat Treatment on Light Transmittance

Light transmissions of aligner materials are influenced by both materials' molecular and crystal structures, and environmental factors such as temperature, humidity, pressure, and heat treatment process.[24,38]

Structurally, polymer materials can be either amorphous or partly crystalline. Amorphous plastics allow visible light to pass through and thus give the materials the transparent appearance. In contrast, semi-crystalline plastics contain mixtures of crystalline and amorphous structures with different refractive indexes. [24] The bundle-like crystalline structure regions in semi-crystalline polymers can scatter visible light to reduce transparency and make the materials more opaque.[48]

Previous studies reported that light transmission of aligners could be affected by oral temperature, food stain and other aging processes during clinical wear. However, the literature data on the effects of heat treatment on aligner light transmittance is lacking. One study investigated the effects of heat treatment on PETG and copolyester aligners.[19] However, the most popular aligners, such as Invisalign, are TPU based. Therefore, it is important to test the results of TPU based materials.

Thermoforming processes can alter the microstructure of thermoplastic materials, affecting the transparency of the materials.[19] In our study, the PETG raw materials showed a greater transparency than TPU-1 (Fig 26) as PETG was mostly amorphous and TPU was semi-crystalline. [24] After heat treatment, TPU-1 had higher light transmission than raw TPU-1. The reduction is partly due to TPU-1 crystalline bundles turning into amorphous structures. Ryokawa et al. reported that the polymer chains can breakdown during heat treatment process, and thus the average molecular weight of the material can reduce which lead to lower thickness.[24] Interestingly, the light transmission rate of thermoformed PETG decreased only slightly compared to PETG raw materials, possibly due to altered polymer chain conformation in PETG during heat treatment. In addition, transparency is found to be associated with reduced thickness.[19,50] TPU-1 showed largest thickness reduction compared to PETG, as shown in previous sections. Therefore, the reduced thickness may also contribute to the increased light transmittance after heat treatment.

During clinical use, the light transmittance may be further modified. Occlusal forces from chewing and bruxism are found to contribute to the opacity of the aligners.[18]

IV 2i. Effects of Heat Treatment on Crack Resistance

After heat treatment, the crack resistance reduced significantly in both TPU-1 and PETG compared to their raw materials (Fig 27).

The reduced crack resistance is directly related to the shrinkage of thickness after heat treatment.[7] In addition, heating temperature above Tg of these materials may have contributed to the fragmentation of polymeric structures and deformation of crystal components during heat treatment.[24]

IV 2j. Effects of Heat Treatment on Microhardness

The heat treatment significantly reduced the microhardness of TPU-1 and PETG (Fig 28, A). Our results are consistent with the results with crack resistance, stress relaxation and glass transitioning changes after heat treatment. Ryu et al. suggested that the hardness difference and surface profiles might be explained by alteration of the polymer crystallinity structures.[19] It is possible that heat treatment resulted in breakdown of polymer chains as suggested by Kown et al.,

[21] which compromises the structural integrity and conformational cohesiveness of materials that lead to reduced surface resistance. [56]

Within the thermoformed aligners, TPU-1 and PETG had similar Vickers hardness. However, the microhardness of TPU-1 and PETG aligners were significantly higher than TPU-2 (Fig 28, B). The results suggest TPU-2 was most susceptible to penetration from heavy impacting forces during chewing and clenching. TPU-1 had the largest resistance against such forces.

IV 3. Aim 3

Aligner materials can absorb water through ambient air humidity or immersion in saliva during intraoral use.[23,60] Water absorption phenomena are caused by attachment of water molecules on the material surface and their subsequent absorption or binding into materials. Water absorption can lead to material swelling that alters the dimension changes of aligner materials.[34,44] In addition, water absorption affects the molecular structures of aligner material. Water absorption can induce structural degradation[25,59] and chemical hydrolysis in polymers,[53] In addition, the process of water absorption can facilitate the attachment of the pigments and bacteria to the material surfaces,[2,5] leading to staining and bacterial plaque accumulation. Oral enzymes may also degrade the structures via enzyme-catalyzed hydrolysis

The material swelling and structural degradation can impair the mechanical properties of aligners [25,51] and lead to compromised the clinical outcomes.[59] The hygroscopic expansion can cause misfit of aligners,[24] so aligners cannot adapt well to tooth surface to produce correct force/moment ratio to produce designated dental movements.[2] Other studies have reported that the tensile strength,[7,34] elastic modulus,[7] delivered force[34,45] of polymers were reduced after soaking in water. This degradation was generally related to the moisture ingress of water into bulk material.[34,45] In addition, the tribological properties of the material surfaces, such as scratching and cracking, were similarly worsened by water soaking process.[34,45] However, there is a lack of data on how water immersion can change the mechanical properties in aligners used in real world. In this section, we addressed this issue and provide data on how water soaking can impair stress relaxation, creep, crack resistance and microhardness and Arrhenius energy of both raw materials and aligner materials.

IV 3a. Effects of Water Absorption on Stress Relaxation

Only a few studies have investigated the stress relaxation of aligner materials after water absorption under intraoral temperature.[7,19,26,34] However, these studies used either raw materials or thermoformed aligners that were impressed on artificial objects rather than patient teeth[7] and thus their clinical implications are limited. As far as the author's knowledge, there are no studies have examined how water affect stress relaxation in aligners used in real world. The complex geometry of teeth may significantly affect the mechanical properties of aligners.[21] Our study provided some insights on stress relaxation in actual aligners.

In our study, DMA was used to quantify stress relaxation of PETG, TPU-1 and TPU-2 materials before and after soaking in PBS (Fig 29-31). Before soaking in PBS buffer, TPU-1 aligners had the highest initial stress followed by PETG and TPU-2 (Table XXIV). After soaking in PBS buffer, the initial stress of all three materials declined. Following 1 week of water absorption, the initial stress or the force exerted by TPU-1 reduced to 70% while PETG and TPU-2 reduced to 80-85% of their original stress levels before soaking. TPU-1 had the largest incremental change in stress relaxation rate after 1 week of soaking as it rose from 78.52 ± 3.41 % to 87.29 ±3.11% (p=0.02). The increase in stress relaxation for PETG and TPU-2 after 1 week of soaking was not statistically significant (p=0.31, p=0.41).

The changes in stress relaxation after water immersion are consistent with the previous results on water absorption and hydrophilicity (see section IV 2d and 2e). TPU-1 had higher water absorption, volumetric swelling and hydrophilicity than PETG. Thus, the high water absorption rates may have led to higher stress relaxation and greater reduction of initial stress in TPU-1 after soaking. PETG and TPU-2 had half of the water absorption of TPU-1 and thus were less susceptible to changes in stress relaxation.

A few studies found that water modify stress relaxation of plastic materials.[7] [34,56] It is thought that water molecules can act as plasticizers that decreases the Tg-values of plastics.[34,52,61] Water molecules are inserted into to macromolecules and structural backbones via hydrogen bonds. They act like as spacers between chains [62,63] which increase the flexibility and the mobility of the chains within the free volume of the amorphous phase of a material. [64] In addition, water can mediate hydrolytic degradation.[26] The backbone chains of aligner materials can be irreversibly degraded by hydrolysis.[45,65] It is also reported that absorbed water may dissolve soluble substances in TPU polymer coatings which accelerates degradation processes.[66] Therefore, the increase in stress relaxation in our tested materials can be caused by the hydrolysis and plasticization effects.

It is reported that the effect of water on mechanical degradation is more pronounced in materials with a higher diffusion rate of hygrothermal expansion.[34] In our study, we found that TPU-1 had higher swelling than PETG. Therefore, our stress relaxation findings are consistent with hygrothermal expansion results. TPU-1 had more absorption of water and thus was more subject to the performance degradation from water compared to the other two materials.

IV 3b. Effects of Low pH Citric Acid on Stress Relaxation

pH plays an important role in modulating the properties through catalysis in polymers.[59] For example, the chemical reaction rates of esters may change at several orders of magnitude from catalysis of hydrogen ions.[25]

Interestingly, TPU-1 thermoformed samples that were soaked in both pH 3 (Fig 32) or pH 5 (Fig 33). The stress relaxation went from 78.20 ±2.11% before soaking to 55.63 ±1.21% in pH 3 buffer and 54.21 ±3.18 % in pH 5 buffer after 1-week soaking (Table XXV and XXVI). The low pH may have accelerated the polymer chain arrangement into an improved amorphous microstructure, preventing the loss of forces delivered by the materials.[59]

The stress relaxation of the samples soaked in pH 3 or pH 5 medium after 1 week were very similar after 7 days (p=0.33) (Table XXV and XXVI). The remaining stress was the same for day 7 soaked and day 7 dried samples, indicating the change of material properties was irreversible after soaking in lower PH medium.

There may be several possible explanations on why citric acid soaking caused reduced stress relaxation of TPU-1 aligner materials. A few studies suggest that citric acid can react with PEG glycol group and undergo esterification and become incorporated into the structural backbone of TPU.[54,67]. The resulted structures provide superior thermostability, increased tensile strength and more resistance to time dependent degradation[67]. Moreover, citric acid can be oxidized with water molecules and form citric acid ester[54], which may act as a stabilizing plasticizer that increase the mechanical properties of aligner[68]. In the future, we will add buffer to citric acid to neutralize pH to rule out the effect of pH and study how citric acid cause such effects. In addition, different acid buffers with different pH will be examined to evaluate their effects on stress relaxation.

IV 3c. Effects of Water Absorption on Microhardness

In our study, we found that the microhardness reduced in both raw and thermoformed materials of PETG and TPU-1 after PBS immersion for 1 week (Fig 34).

Previous studies have also found that water ingression can irreversibly change the wear resistance of plastic materials. Boubakri et al. found that the water immersion can increase the wear rate of thermoformed polyurethane materials.[34] Guermazi et al. reported that the hygrothermal aging process weakened wear and penetration resistance of the polymer coating.[34] The reduced abrasion resistance can be explained by the plasticization effect resulted from penetration of solvent molecules into the polymer structure which generates degradation of the polymer structure. Therefore, our results are consistent with these previous findings.

IV 3d. Effects of Water Absorption on Crack Resistance

The crack resistance of TPU-1 raw and thermoformed materials decreased significantly after soaking in PBS for 1 week (Fig 35). However, the crack resistance of PETG raw and thermoformed materials did not change significantly after PBS soaking.

Previous studies found that water immersion can reduce material resistance of plastics due to the penetration of water molecules into the polymer structure.[7] Water can cause degradation of internal polymer structures and result in irreversible impairment of structural integrity and ability to resist external mechanical impact forces.[34,56,69] Ingression of water may also induce stress between amorphous (relatively water accessible) or crystalline (relatively water impenetrable) zones. The water induced stress can create structural disorder that weakens and even destroys the secondary links between chains responsible for the material cohesion.[27,58] The combination of mechanical cohesion and increased the molecular mobility can contribute to the increased susceptibility to crack. [34,56,69] [58] [27]

In our study, TPU-1 had more water absorption and volume expansion from PBS immersion. Thus, it is possible that the increased water content led to more structural alternation or degradation which contributed to increased crack resistance. This result in good accordance with previous studies on the effect of water on polyurethane materials. [34,58,61]

IV 3e. Effects of Water Absorption on Creep

After soaking in PBS for one week at 37 °C, the creep modulus of all three thermoformed aligner materials increased significantly ($p<0.05$ for all three materials) (Table XXVII). Our data suggest that all three materials became more susceptible to deformation after PBS immersion. These results are consistent with our findings of Arrhenius activation energy, where the energy values of soaked materials were 7-15 KJ/mol smaller compared to the materials in room humidity.

Our findings are also congruous with other studies. Airoldi G et al. and Azhikannickal E et al. also found that water storage can lowering the creep resistance of the PETG materials.[34,58] Previous studies have attribute this phenomenon to the plasticization effect of water molecules. Water ingression can reduce the mechanical cohesion and increase the mobility of the polymer chains, [34,58] which increase the likelihood of a material to undergo creep.

The creep compliance of PETG and TPU-2 increased by around 45% after PBS soaking, while that of TPU-1 increased by 66% (Table XXVIII). The creep compliance values of all three materials are consistent with the previous data on water absorption. TPU-1 had the largest water absorption, volume expansion and hydrophilicity, while PETG and TPU-2 had similar smaller values (see section IV 2d and 2e). Thus, the high material swelling, and absorption may predispose the TPU-1 material to be more susceptible to the degradation effect of water absorption. As a result, TPU-1 demonstrated more creep compared to others.

IV 3f. Effects of Water Absorption on Arrhenius Energy

Before soaking in PBS, TPU-2 thermoformed aligners had the highest Arrhenius energy, followed by TPU-1 and PETG (Table XXIX). After soaking in PBS, the Arrhenius energy of all materials reduced by 7-15 KJ/mol compared to the materials that were not immersed in PBS. The reduction of activation energy in TPU-2 aligners is significant ($p=0.04$).

The reduced Arrhenius energy can be used to explain why all three materials became less resistant to time-dependent mechanical and thermal deformation after soaking in PBS. These data are consistent with our findings on the mechanical degradation of stress relaxation, creep, microhardness and crack resistance.

V. STUDY LIMITATIONS AND FUTURE DIRECTIONS

The first limitation of the study is that we characterized the mechanical properties of aligner materials from one structural orientation. The aligner samples that were used in stress relaxation and creep testing were obtained from the lingual portions of central incisors, and stress and strain were applied to samples from incisogingival direction. However, the structural orientation within the same aligner can vary depending on the location within the aligner as various orientations may confer different physical properties within the aligner material.[24] Therefore, in future studies, we will characterize the molecular and structural orientations as well as the mechanical properties of aligners in various directions.

The second limitation of this study is that the data on mechanical properties are empirical in nature and may not fully translate into clinical performance. We showed that all three aligner materials experienced significant stress relaxation. However, it is unclear if the remaining force after relaxation is insufficient or excessive for orthodontic movement. The only way to verify the force level in vitro is to mount an aligner in an artificial dental model with individual tooth attached to a multiaxial force gauge, similar to what Elkholy et al. described in their study.[37] However, Elkholy et al. only tested one tooth with a uniaxial force gauge, and they did not quantify stress relaxation in their study. In addition, studies showed that periodontal ligament (PDL) had viscoelastic properties.[70,71] PDL may also undergo force decay like aligners which can further complicate the force delivery results. Our future studies will establish a multiaxial force gauge system to simulate the orthodontic forces exerted on each tooth from the aligners with an experimental and computational dynamic method. In vivo studies can be carried out to account for the effect of PDL on force delivery.

Lastly, in addition to water, the bacteria and enzymes present in saliva may affect the mechanical properties of aligners. Bacteria can produce byproducts such as enzymes and other proteins that may modify properties of aligners such as glass transitioning temperature and tensile strength.[51] In future studies, we will evaluate how oral enzymes and bacterial species affect aligner properties.

VI. CONCLUSION

In the current study, we developed a comprehensive protocol to characterize themomechanical properties for both raw and thermoformed aligner materials. Using this protocol, we quantified measurements for mechanical properties that were consistent with the structural and thermal properties, namely the crystallinity and Arrhenius energy. The protocol was also used to quantify changes in mechanical properties after heat treatment and water absorption, and the results corresponded to changes in crystallinity and Arrhenius energy. The following findings were derived from the experimental data:

(i) Aligner materials had varying chemical compositions and physical structures that conferred different mechanical properties. The crystallinity in particular played an important role in the dictating their properties. Our findings showed that the semi-crystalline TPU-1 had higher Tg and microhardness, more resistance to crack and creep, and less light transmittance compared to amorphous PETG. These differences in mechanical property were consistent with our crystallization characterization results.

(ii) Heat treatment significantly modified the crystallinity of aligners and impaired their mechanical properties. Thickness was reduced considerably after the heat treatment, which weakened multiple mechanical properties that were dependent upon thickness. Heat treatment of TPU-1 caused a shift from a semi-crystalline to amorphous composition, leading to further degradation of properties such as microhardness, crack resistance, stress relaxation and creep compared to amorphous PETG. TPU-1 also increased in light transmittance, hydrophilicity and water absorption as a result of heat treatment.

iii) Water absorption dramatically reduced the crack resistance, microhardness and Arrhenius energy of the aligners. TPU-1 aligners had the most water absorption and material swelling, which can be accounted by the increased structural vacancy from crystal breakdown from heat fabrication. Therefore, TPU-1 was more susceptible to mechanical degradation from water-mediated hydrolysis than PETG and TPU-2.

iv) Within a single aligner, the thicknesses and structural orientation are not distributed evenly, resulting in differing properties within the same aligner. There is a large variation of aligner thicknesses depending on the complex anatomy of the dental morphology and the distance of the material that is being stretched from the original dimension to the final fabricated aligner. In addition, the aligner may have different structural orientations based on manufacturing processes. For accurate characterization, the mechanical properties should be characterized from multiple locations and orientations.

Due to the limitations of current aligner materials, the future direction of aligner material development should focus on 1) reducing the time-dependent mechanical decay such as creep and stress relaxation, 2) minimizing the effects of heat treatment with crystal stabilizers, 3) reducing the plasticizer effects of water on mechanical properties by modifying polar groups and control material swelling, 4) reducing the discrepancy in thickness distribution with better heating protocols or 3D printing that can precisely control thicknesses, and 5) combining materials with different properties to express differential mechanical properties to provide desirable force/moment ratios for more effective orthodontic tooth movement.

FIGURES AND TABLES

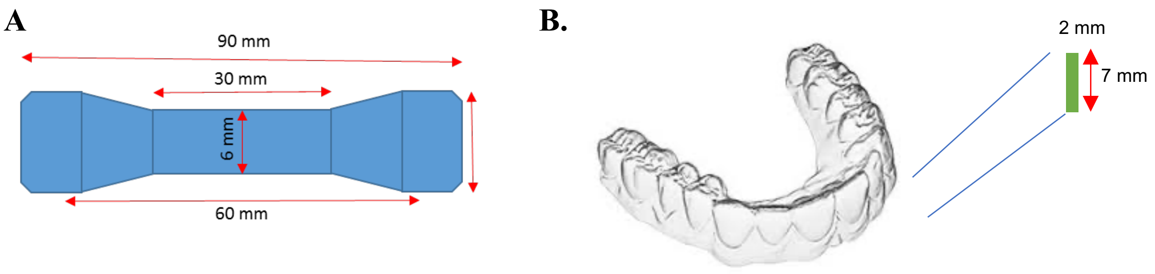

Figure 1. Typical required sample size for **A**. Instron system (90mm X 6mm) and **B.** DMA (7mm X 2 mm). DMA can measure smaller samples from fabricated aligner.

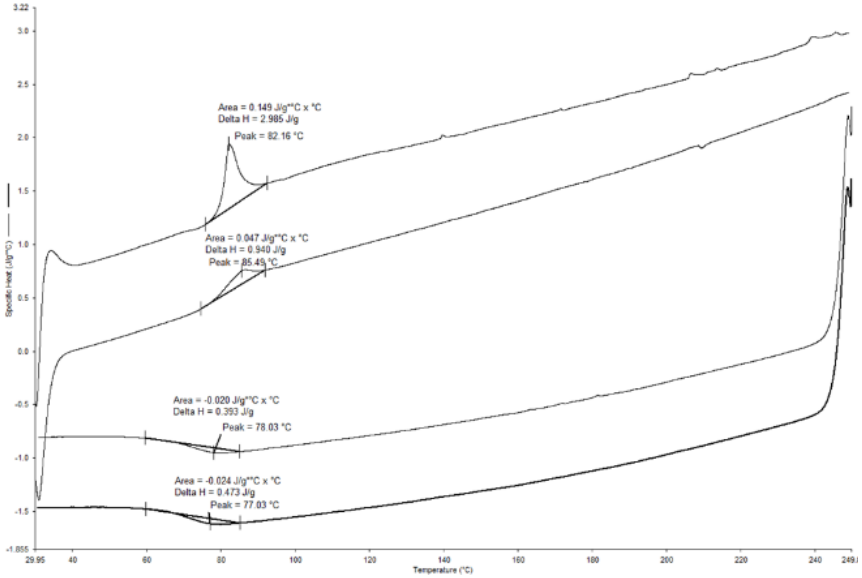

Figure 2. DSC thermographs of PETG raw material.

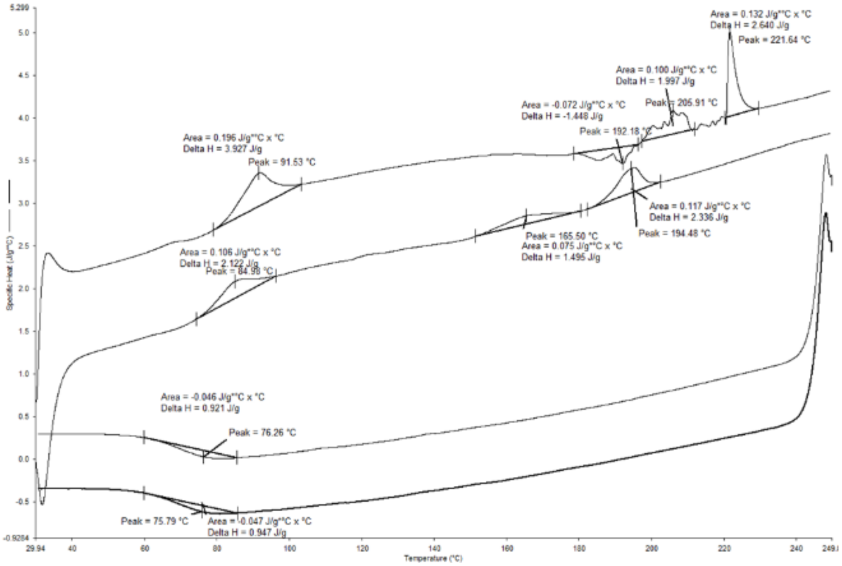

Figure 3. DSC thermographs of TPU-1 raw material.

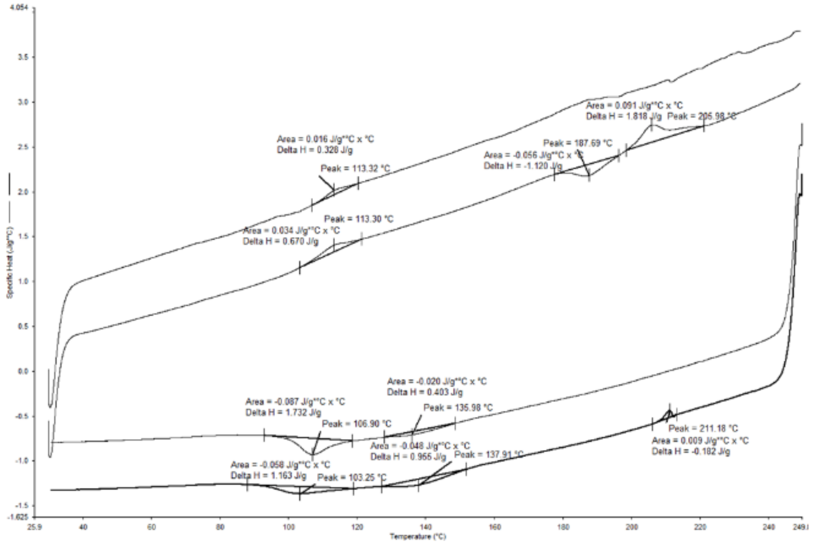

Figure 4. DSC thermographs of TPU-2 thermoformed clear aligners that were tested as received.

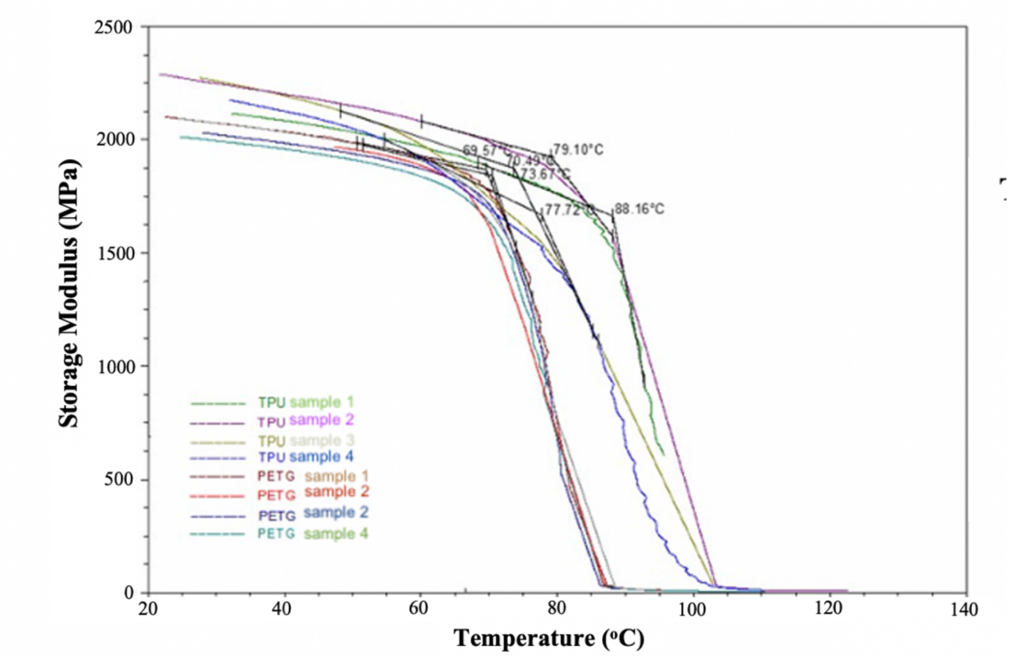

Figure 5. Glass transition temperature (T_g) of raw materials of PETG and TPU-1 measured by DMA.The Tg was determined by the inflection point of the stiffness vs. temperature plot.

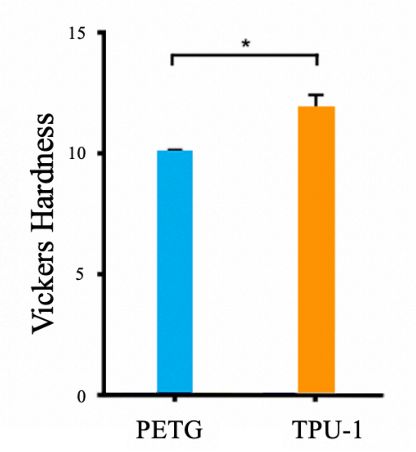

Figure 6. Microhardness (Vickers hardness) of raw materials of PETG and TPU-1.

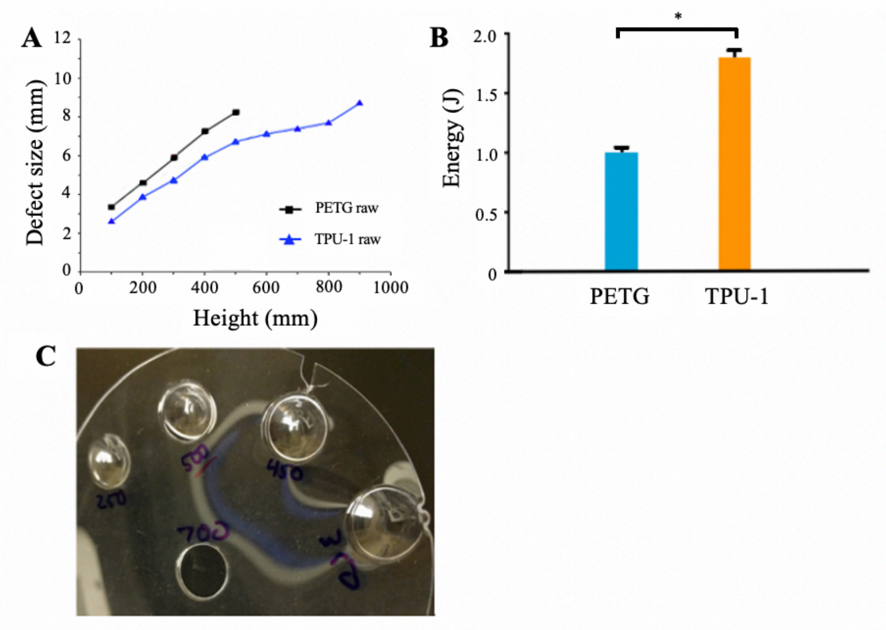

Figure 7. Crack resistance of raw materials of PETG and TPU-1. **A.** Defect size resulted from impact force of the two materials. **B.** Failure energy absorbed by two materials before cracking. **C.** Indentations resulted from the impact force.

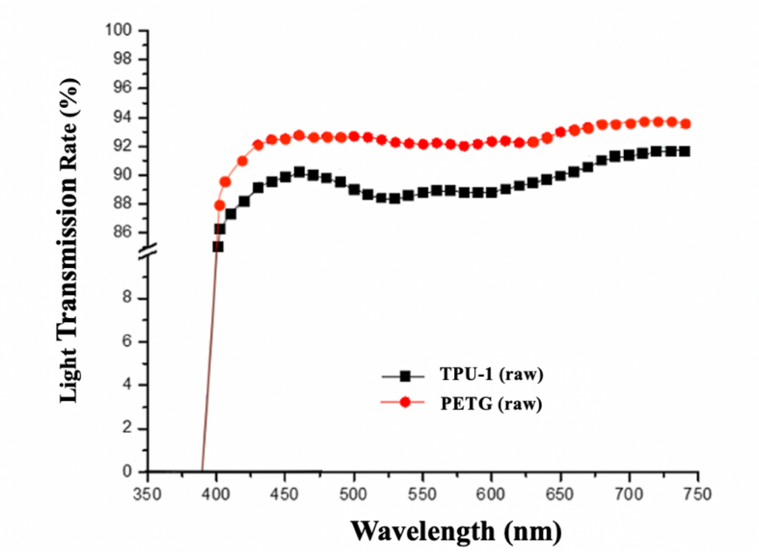

Figure 8. Light transmittance of PETG and TPU-1 raw materials.

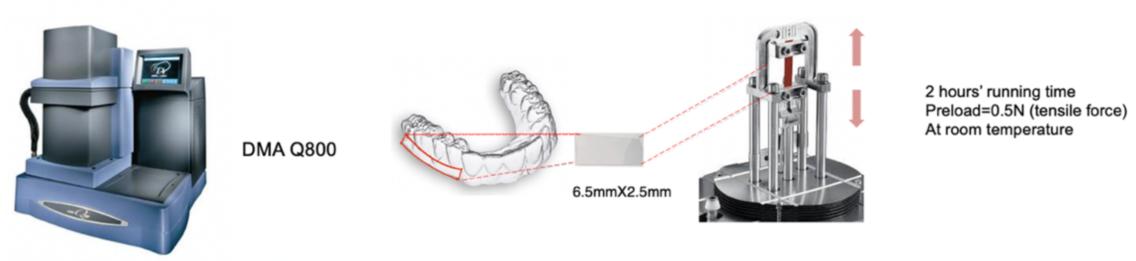

Figure 9. Stress relaxation measured with TA Instruments ® DMA Q800 machine. Samples of either raw sheets or fabricated aligners were cut and loaded onto tensile clamps of DMA machine, and stress relaxation was measured with a preload of 0.5 N.

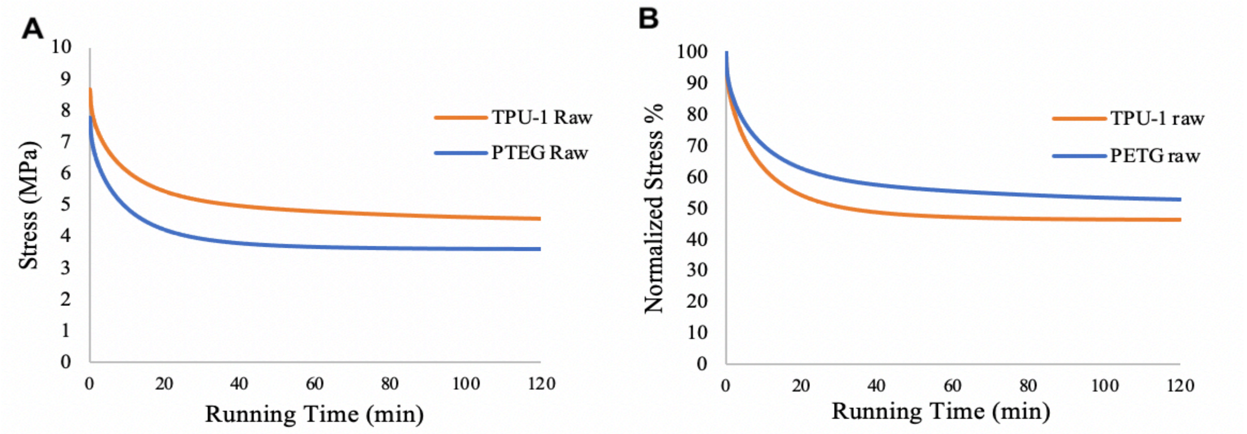

Figure 10. Stress relaxation (initial vs. final stress) of PETG and TPU-1 raw materials measured by DMA. **A.** Stress vs. Time plot for both materials. **B.** Normalized Stress (remaining stress/initial stress) vs. Time plot for both materials.

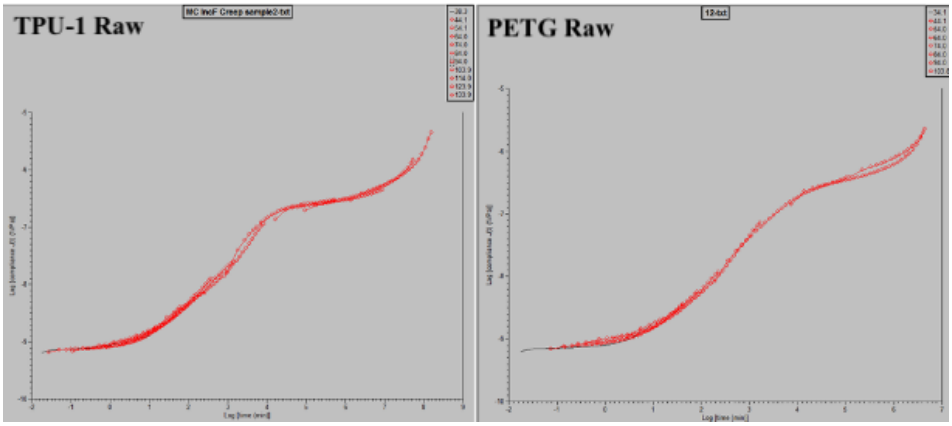

Figure 11. Master Curves of Time-Dependent Superimpositions (TTS) for TPU-1 and PETG raw materials for creep quantification and predictions.

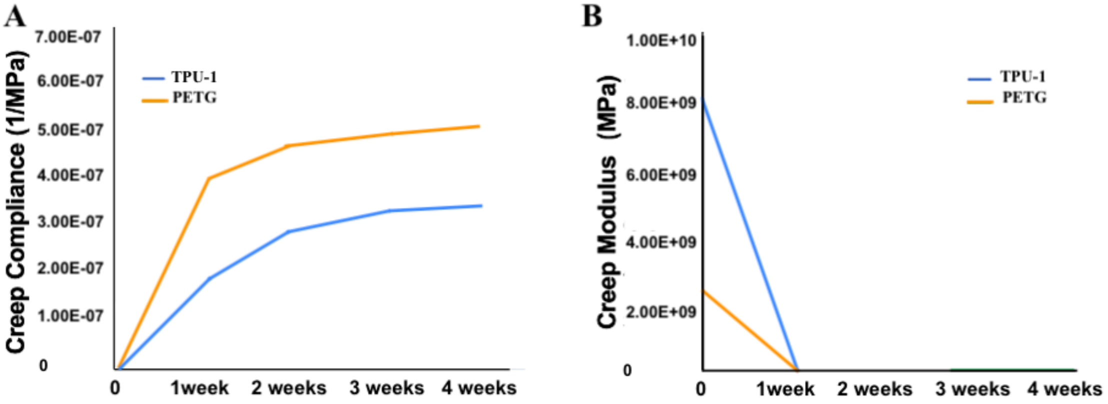

Figure 12. Creep results of PETG and TPU-1 raw materials measured by DMA. **A.** Creep compliance (1/Pa) **B.** Creep modulus (Pa) of both materials.

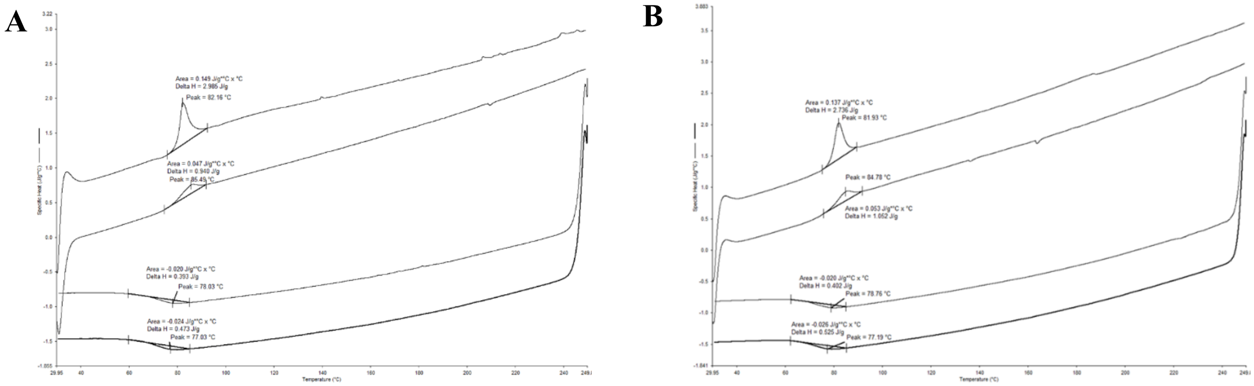

Figure 13. DSC thermographs of PETG samples from **A.** PETG raw material and **B.** PETG clear aligners.

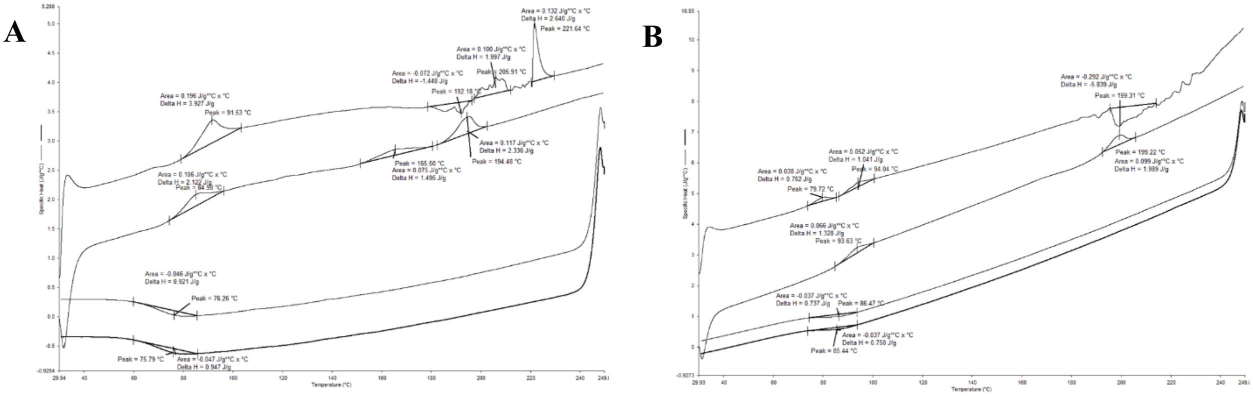

Figure 14. DSC thermographs of TPU-1 samples from **A.** TPU-1 raw material and **B.** TPU-1 clear aligners.

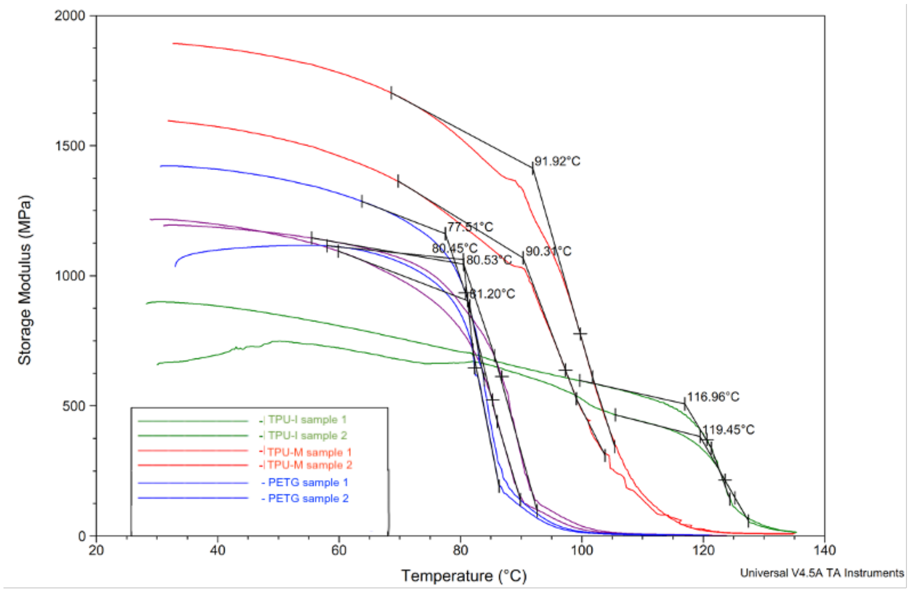

Figure 15. Glass transition temperature (Tg) of PETG and TPU-1 raw materials and aligners, as well as TPU-2 aligners. The Tg was determined by the inflection point of the stiffness vs. temperature plot measured from DMA.

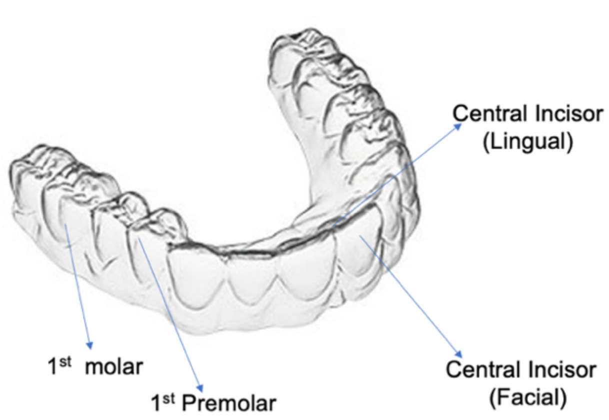

Figure 16. The thickness of aligners measured at three locations: central incisor, first premolar and first molar.

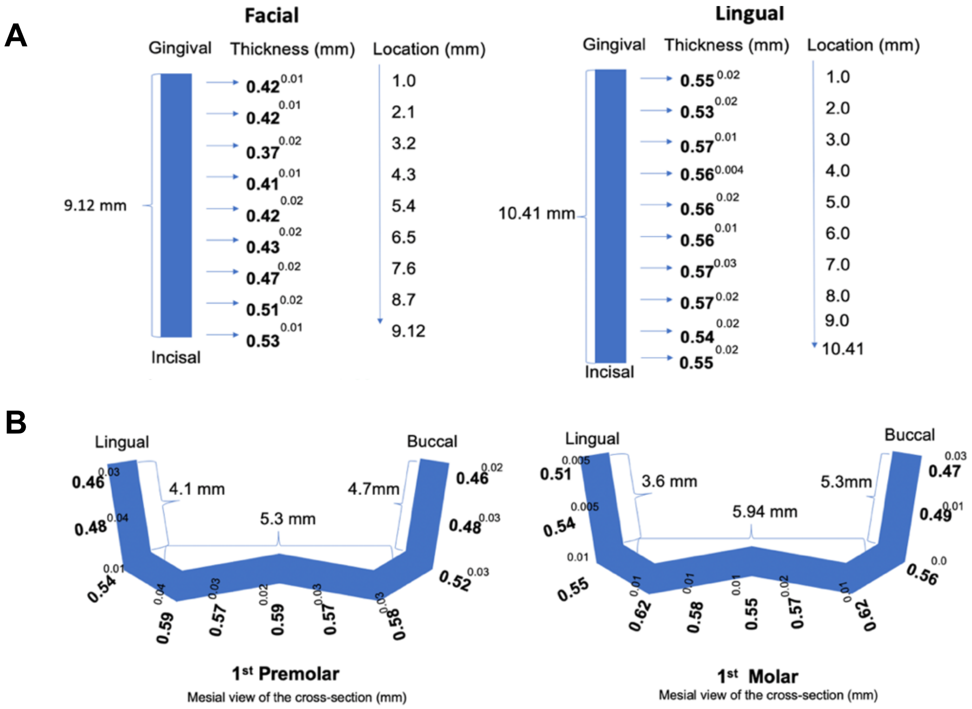

Figure 17. The thickness of PETG aligners was measured from **A.** central incisor (facial and lingual) **B.** 1st premolar and 1st molar regions.

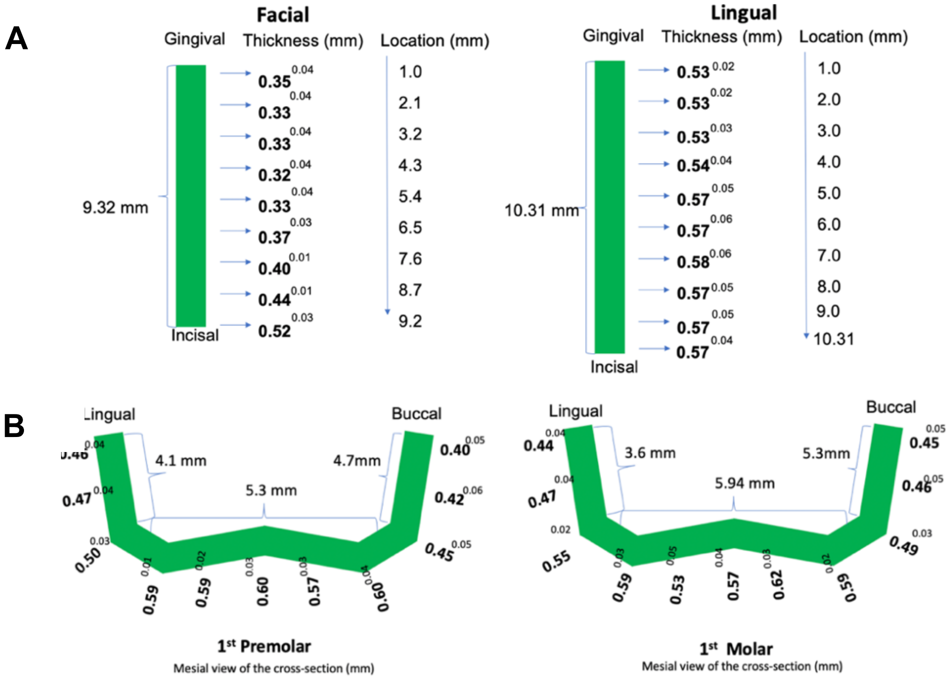

Figure 18. The thickness of TPU-1 aligners was measured from **A.** central incisor (facial and lingual) **B.** 1st premolar and 1st molar regions.

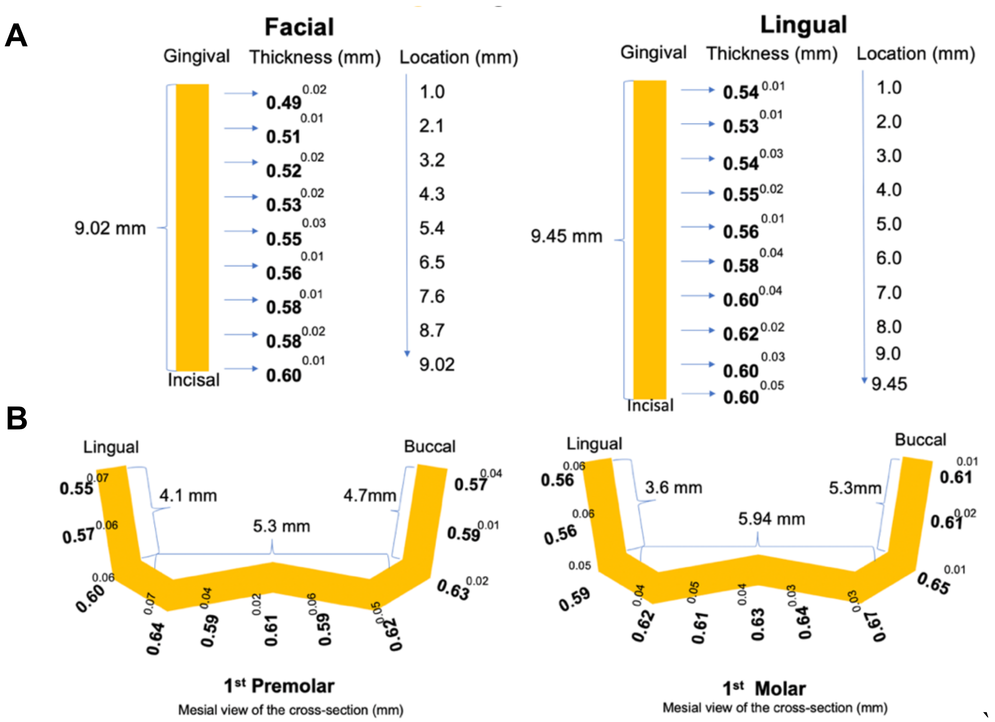

Figure 19. The thickness of TPU-2 aligners was measured from **A.** central incisor (facial and lingual) **B.** 1st premolar and 1st molar

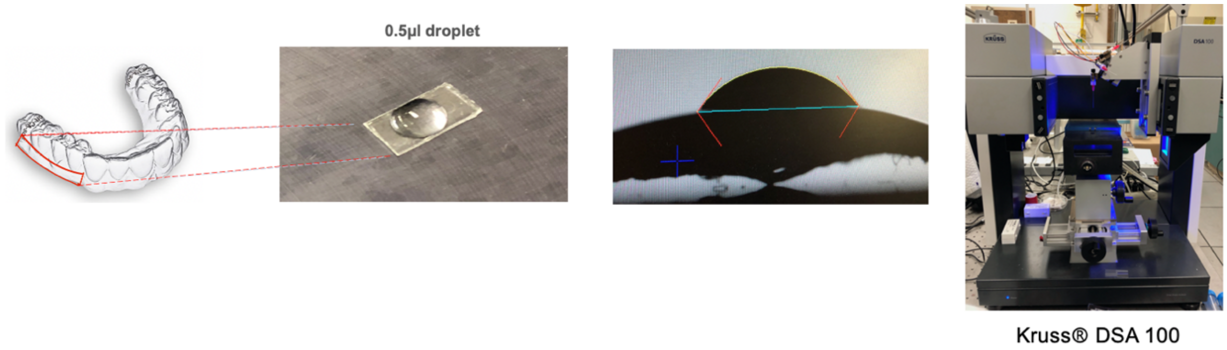

Figure 20. Measurement of hydrophilicity through contact angle. 0.5 µl deionized water droplet was placed on sample surface. Contact angles were measured with Kruss® DSA 100.

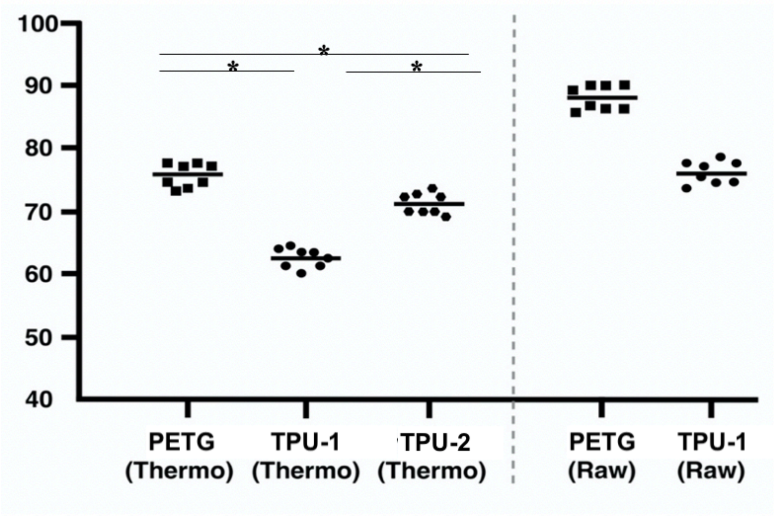

Figure 21. Measurement of contact angles to assess the hydrophilicity of PETG and TPU-1 raw materials; and PETG, TPU-1 and TPU-2 aligners.

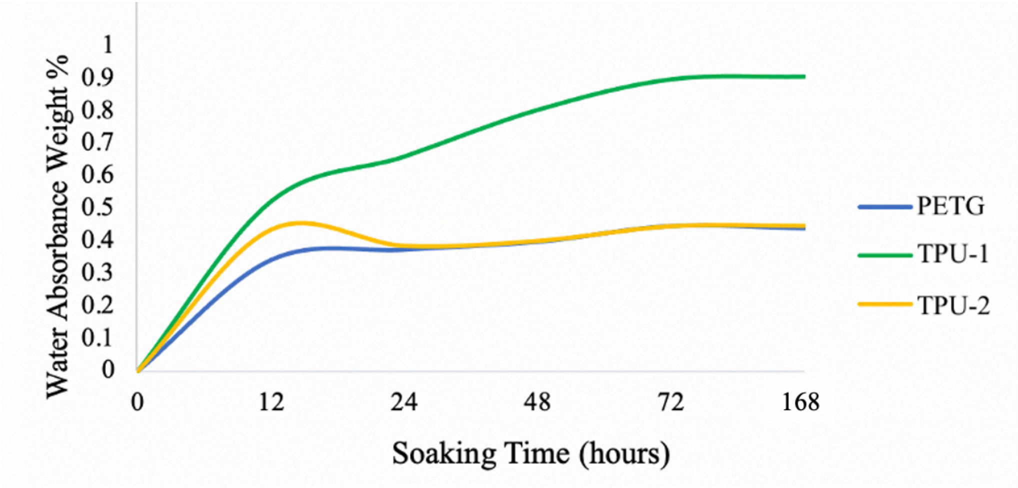

Figure 22. Water absorption rates of aligners that were soaked in PBS medium at different timepoints.

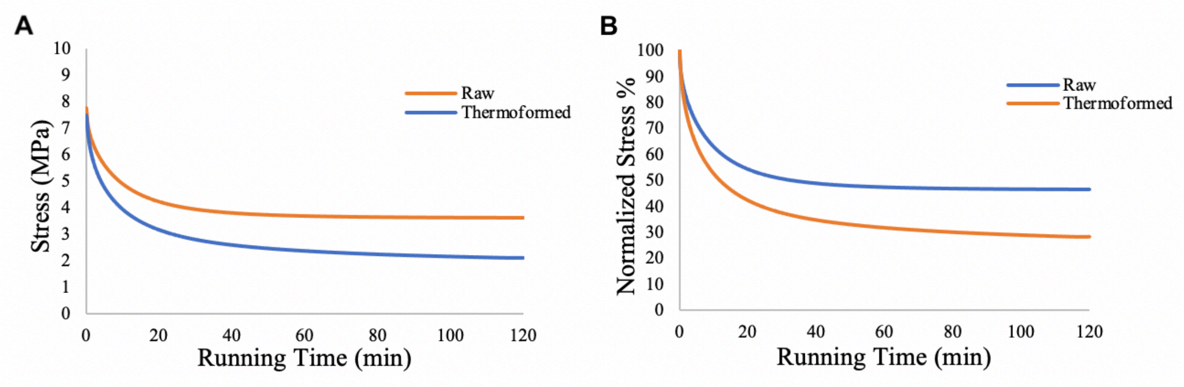

Figure 23. Stress relaxation (initial vs. final stress) of PETG raw materials and aligners measured by DMA. **A.** Stress vs. Time plot for both materials. **B.** Normalized Stress (remaining stress/initial stress) vs. Time plot for both materials.

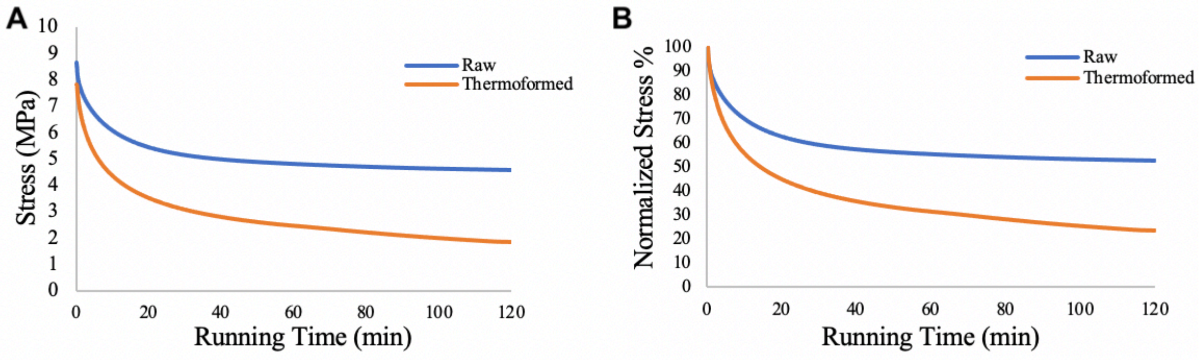

Figure 24. Stress relaxation (initial vs. final stress) of TPU-1 raw materials and aligners measured by DMA. **A.** Stress vs. Time plot for both materials. **B.** Normalized Stress (remaining stress/initial stress) vs. Time plot for both materials.

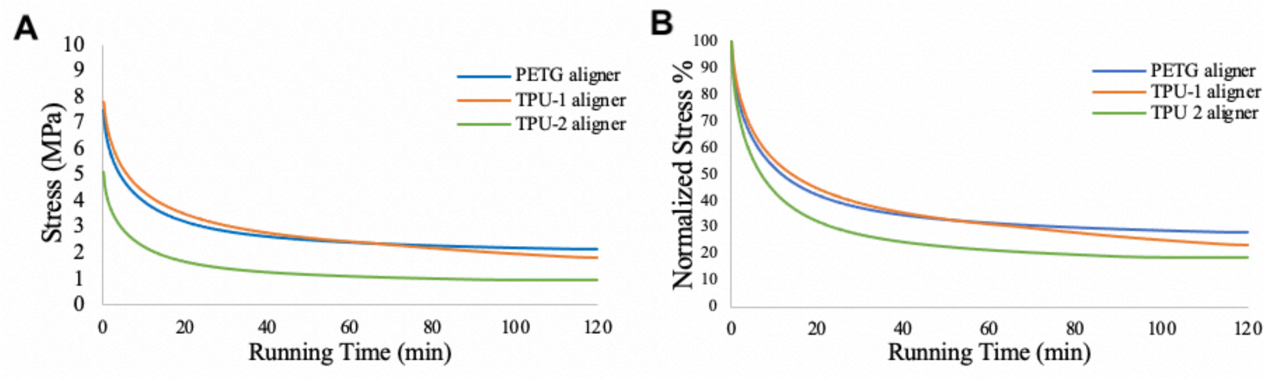

Figure 25. Stress relaxation (initial vs. final stress) of three aligners measured by DMA. **A.**Stress vs. Time plot for both materials. **B.** Normalized Stress (remaining stress/initial stress) vs. Time plot for both materials.

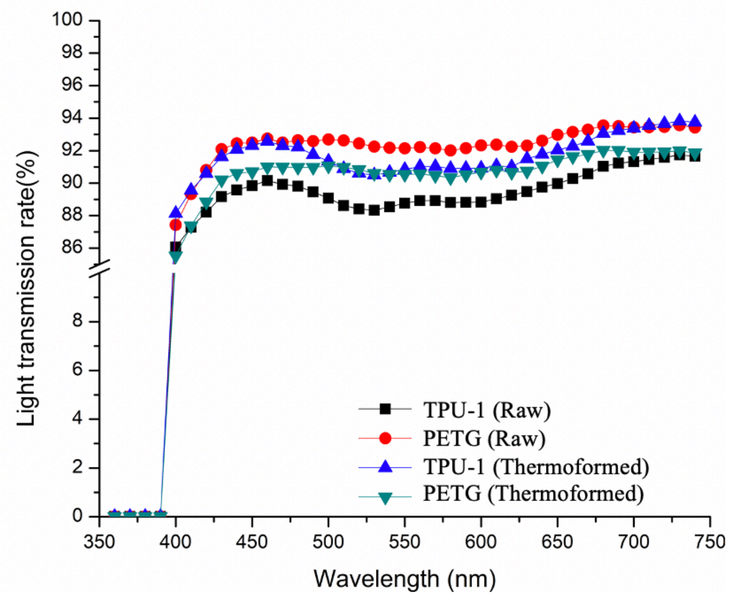

Figure 26. Light transmittance of PETG and TPU-1 raw and thermoformed materials.

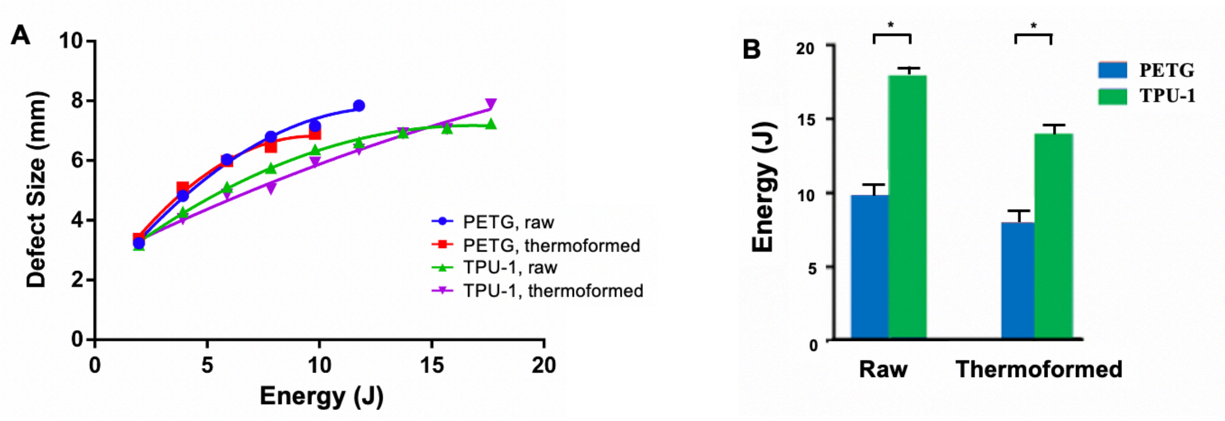

Figure 27. Crack resistance of raw and thermoformed materials of PETG and TPU-1. **A.** Defect size resulted from impact force of the materials. **B.** Failure energy absorbed by two materials before cracking.

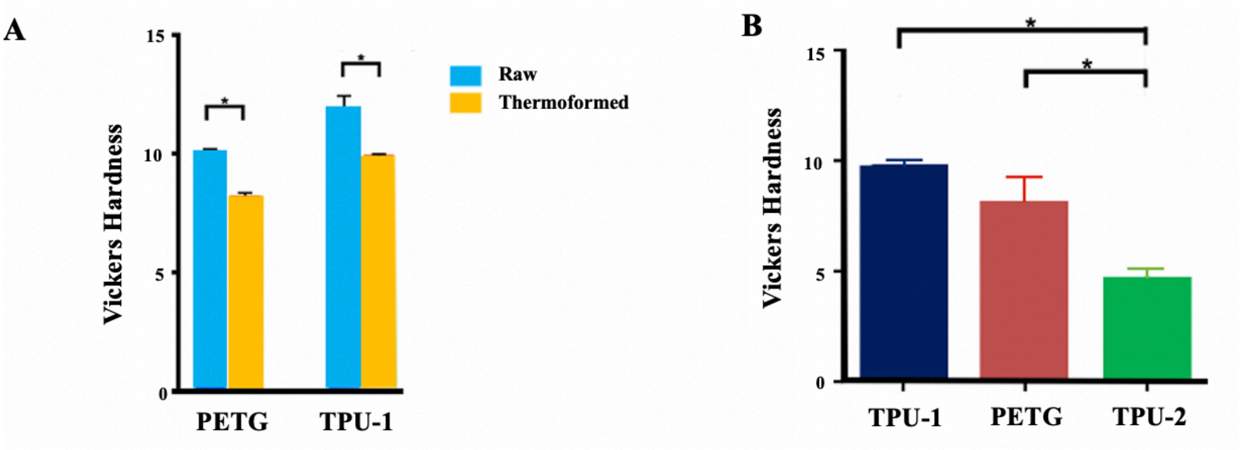

Figure 28. Microhardness of different materials. **A.** PETG and TPU-1 raw materials and thermoformed aligners (n=6) **B.** PETG, TPU-1 and TPU-2 thermoformed aligners (n=6).

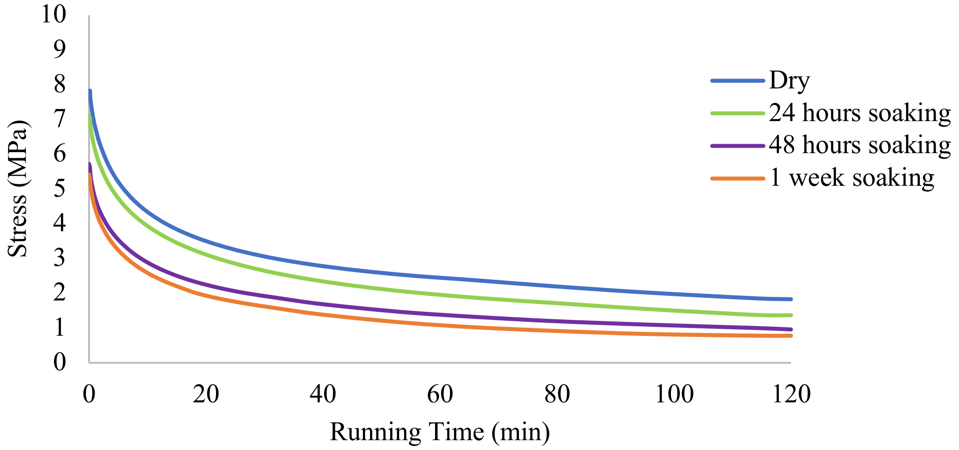

Figure 29. Stress relaxation of TPU-1 aligners that were soaked in PBS at different time points.

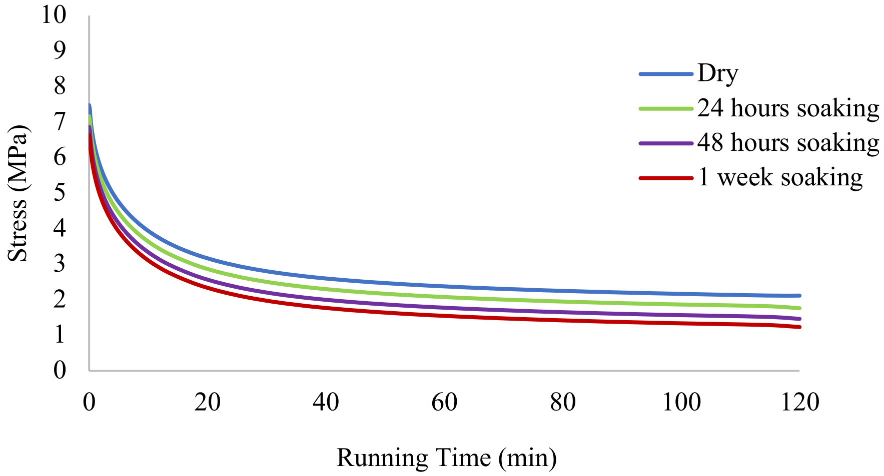

Figure 30. Stress relaxation of PETG aligners that were soaked in PBS at different time points.

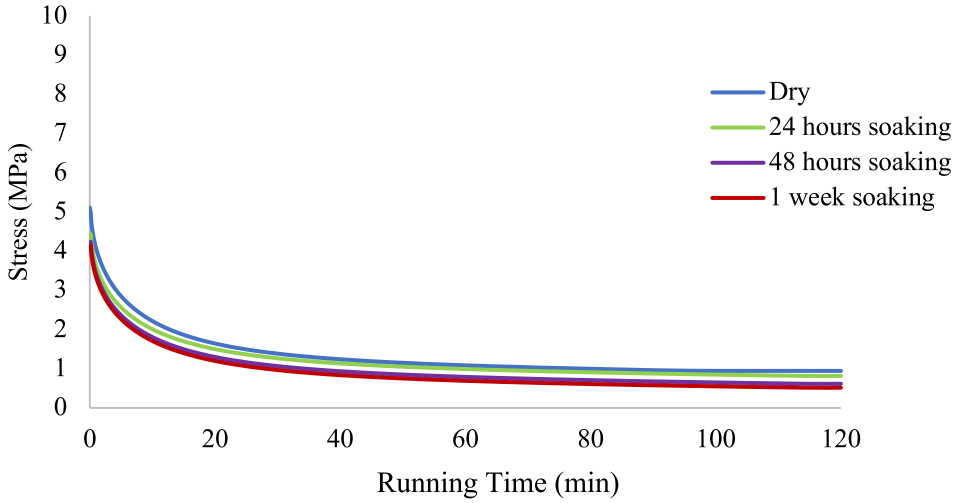

Figure 31. Stress relaxation of TPU-2 aligners that were soaked in PBS at different time points.

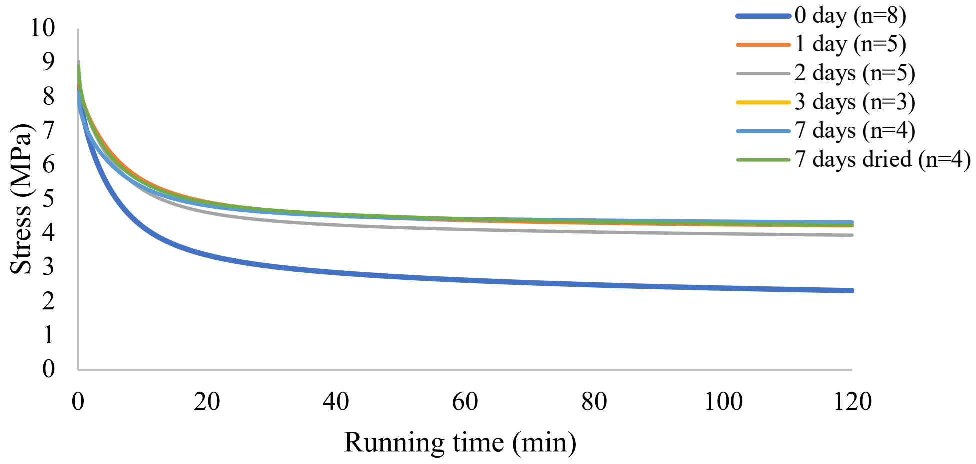

Figure 32. Stress relaxation of TPU-1 aligners that were soaked in Na_2HPO_3/citric acid buffer solution with a pH of 3 at different time points.

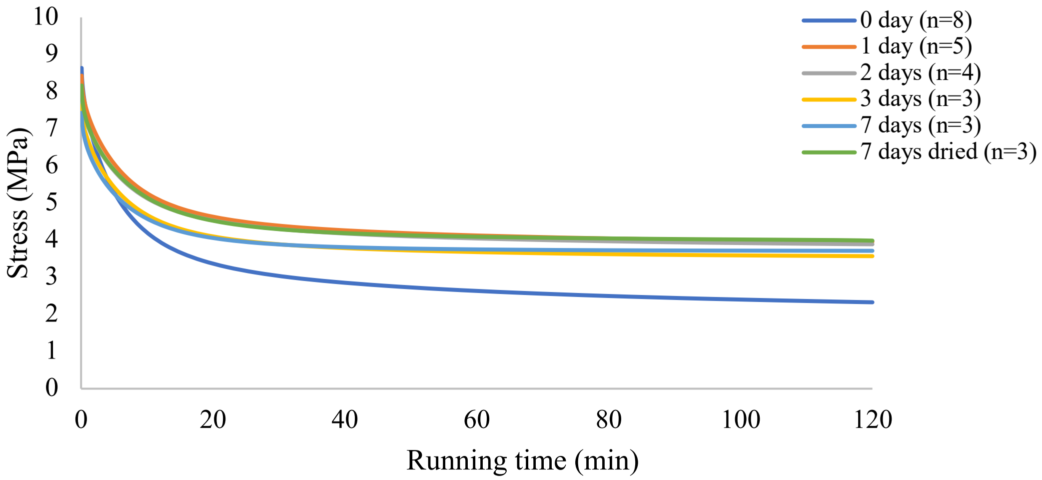

Figure 33. Stress relaxation of TPU-1 aligners that were soaked Na_2HPO_3/citric acid buffer with a pH of 5 at different time points.

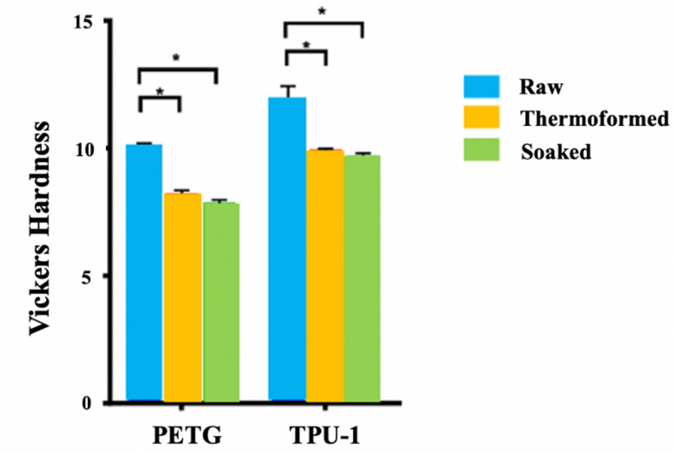

Figure 34. Microhardness of PETG and TPU-1 aligners soaked in PBS for 1 week compared to the dry thermoformed materials and their raw materials.

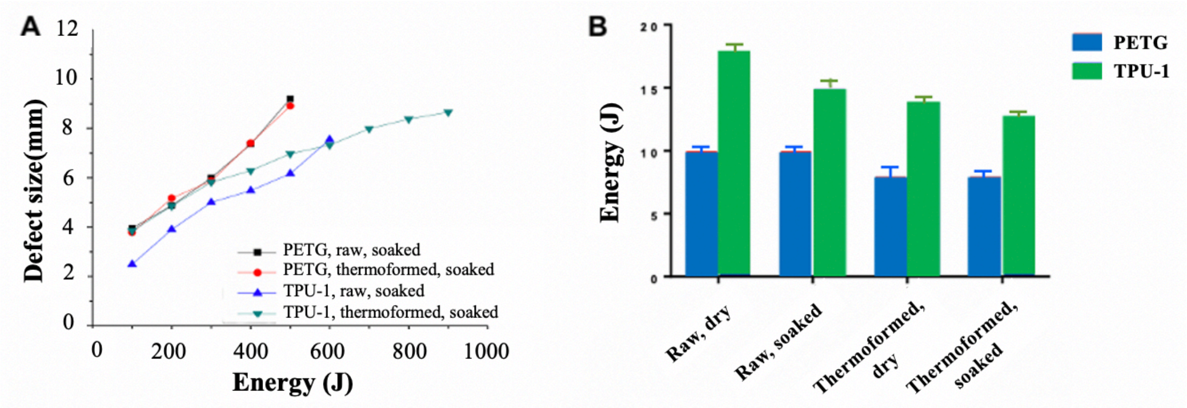

Figure 35. Crack resistance of PETG and TPU-1 raw and thermoformed materials before and after soaking in PBS for 1 week.

Table I: List of tested raw and thermoformed materials with chemical compositions.

Conditions	Materials	Composition
Raw Material Sheets	PETG	Polyethylene terephthalate glycol
	TPU-1	Thermoplastic polyurethane
Thermoformed Aligners	PETG	Polyethylene terephthalate glycol
	TPU-1	Thermoplastic polyurethane
	TPU-2	Ethylene diphenyl diisocyanate, 1,6-hexanedial and additives[24]

Table II. Glass transition temperature (T_g) of raw PETG and TPU-1 measured by DMA.

Raw materials	T_g (°C) [a]
TPU-1	80.29 ± 2.73
PETG	68.60 ± 2.19

[a]. Tg was determined by the inflection point of the stiffness vs. temperature plot.

Table III. Stress relaxation (initial vs. final stress) of raw PETG and TPU-1 measured by DMA

Material	Conditions	Initial Stress at t=0 h (MPa)	Final Stress at t=2 h (MPa)	Stress Relaxation (%)	Relaxation Velocity (MPa/h)
PETG	Raw (n=6)	7.78 ±0.43	3.91 ±0.39	49.74% ±3.21%	1.94 ±0.22
TPU-1	Raw (n=6)	8.66 ±0.51	4.32 ±0.50	51.20% ±2.03%	2.17 ±0.31

Table IV. Creep compliance (1/Pa) of PETG and TPU-1 raw materials from week 0 to week 4.

Material	Compliance 0 week (1/Pa)	Compliance 1 week (1/Pa)	Compliance 2 weeks (1/Pa)	Compliance 3 weeks (1/Pa)	Compliance 4 weeks (1/Pa)
PETG	3.87×10^{-10}	4.03×10^{-7}	4.59×10^{-7}	4.75×10^{-7}	4.98×10^{-7}
TPU-1	1.24×10^{-10}	1.78×10^{-7}	2.79×10^{-7}	3.10×10^{-7}	3.24×10^{-7}

Table V. Creep modulus (Pa) of PETG and TPU-1 raw materials from week 0 to week 4.

Material	Modulus 0 week (Pa)	Modulus 1 week (Pa)	Modulus 2 weeks (Pa)	Modulus 3 weeks (Pa)	Modulus 4 weeks (Pa)
PETG	2.58×10^{9}	2.48×10^{6}	2.18×10^{6}	2.11×10^{6}	2.01×10^{6}
TPU-1	8.09×10^{9}	5.62×10^{6}	3.58×10^{6}	3.23×10^{6}	3.09×10^{6}

Table VI. Arrhenius Energy (Ea) of PETG and TPU-1 raw materials.

Aligners	**PETG**	**TPU-1**
Condition	Raw	Raw
Tested number	6	6
Ea (KJ/mol)	403	418
Std (KJ/mol)	3.12	3.77

Table VII. Glass transition temperature of raw and thermoformed materials measured by DMA.

Material	Conditions	Tg (ºC) [a]
TPU-1	Raw	80.29±1.31
	Thermoformed	75.70±0.68
PETG	Raw	68.60±1.21
	Thermoformed	67.46±0.23
TPU-2	Thermoformed	118.2±1.76

[a]. Tg was determined by the inflection point of the stiffness vs. temperature plot.

Table VIII. Thickness of the raw materials of PETG, TPU-1 and TPU-2.

Material	Conditions	Thickness (mm)
PETG	Raw (n=6)	0.76 ±0.03
TPU-1	Raw (n=6)	0.76 ± 0.01
TPU-2	Raw	0.76 [24]

Table IX. Thickness of central incisor of thermoformed aligners of PETG,TPU-1 and TPU-2.

Aligner Material/Brand	Incisor Facial (mm)	Incisor Lingual (mm)	1st Premolar (mm)	1st Molar (mm)
PETG	0.44±0.05	0.56±0.01	0.53±0.05	0.55±0.05
TPU-1	0.37±0.07	0.56±0.02	0.51±0.08	0.52±0.06
TPU-2	0.55±0.04	0.57±0.03	0.60±0.03	0.61±0.03

Table X. Thickness of central incisor of thermoformed aligners of PETG,TPU-1 and TPU-2. in 1st premolar and 1st molar regions.

Material	Tooth	Facial	Lingual	Cervical
PETG	1st premolar	0.47±0.01	0.47±0.01	0.57±0.03
	1st molar	0.48±0.01	0.53±0.02	0.58±0.03
TPU-1	1st premolar	0.41±0.01	0.47±0.02	0.56±0.03
	1st molar	0.46±0.01	0.46±0.02	0.56±0.04
TPU-2	1st premolar	0.58±0.01	0.56±0.01	0.61±0.02
	1st molar	0.61±0.00	0.56±0.00	0.63±0.03

Table XI. Values of contact angle of different aligners

	PETG Thermoformed Aligner	TPU-1 Thermoformed Aligner	TPU-2 Thermoformed Aligner	PETG Raw Material	TPU-1 Raw Material
Equilibrium contact angle (°)	76.67±1.31	61.20±1.3	70.01±2.39	88.01±2.44	76.89±2.31

Table XII. Weight % increase of raw materials before and after soaking in PBS for 1 week.

Materials	Dry	1-week PBS soaking
PETG	0	0.34±0.06 %
TPU-1	0	0.67±0.04 %

Table XIII. Water absorption percentages of aligners that were soaked in PBS medium

Aligners	0 hour	12 hours	24 hours	48 hours	72 hours	1week
PETG	0	0.34±0.04	0.38±0.02	0.40±0.02	0.45±0.03	0.44±0.06
TPU-1	0	0.52±0.07	0.66±0.07	0.80±0.04	0.90±0.05	0.91±0.04
TPU-2	0	0.44±0.09	0.39±0.06	0.40±0.08	0.45±0.09	0.45±0.09

Table XIV. Water absorption percentages of raw materials soaked in PBS medium.

Aligner Raw Material	Thickness (mm) Dry	Thickness (mm) 1-week Soaking	Ratio of after/before water absorption (%)
PETG	0.76±0.01	0.80±0.01	105.3
TPU-1	0.76±0.03	0.82±0.02	107.9

Table XV. Water absorption percentages of aligners soaked in PBS medium for 1week.

Aligner Material	Thickness (mm) Dry	Thickness (mm) 1-week Soaking	Ratio of after/before water absorption (%)
PETG	0.56±0.01	0.62±0.01	110.7
TPU-1	0.56±0.02	0.64±0.01	114.3
TPU-2	0.57±0.03	0.61±0.01	107.0

Table XVI. Stress relaxation of raw materials of PETG and TPU-1.

Material	Conditions	Initial Stress at t=0 h (MPa)	Final Stress at t=2 h (MPa)	Stress Relaxation (%)	Relaxation Velocity (MPa/h)
PETG	Raw (n=6)	7.78 ±0.43	3.91 ±0.39	49.74% ±3.21%	1.94 ±0.22
TPU-1	Raw (n=6)	8.66 ±0.51	4.32 ±0.50	51.20% ±2.03%	2.17 ±0.31

Table XVII. Stress relaxation of thermoformed aligner materials of PETG and TPU-1.

Material	Conditions	Initial Stress at t=0 h (MPa)	Final Stress at t=2 h (MPa)	Stress Relaxation (%)	Relaxation Rate (MPa/h)
PETG	Thermoformed (n=6)	7.47 ±0.63	2.03 ±0.54	72.89% ±3.01%	2.72 ±0.34
TPU-1	Thermoformed (n=6)	7.85 ± 0.62	1.69 ±0.62	78.52% ±3.41%	3.13 ±0.36

Table XVIII. Stress relaxation of aligner materials of PETG, TPU-1 and TPU2 aligners.

Material	Conditions	Thickness (mm)	Width (mm)	Length (mm)
PETG	Raw (n=6)	0.76 ±0.03	2.24 ±0.03	6.93 ±0.04
	Thermoformed (n=6)	0.57 ±0.03	2.21 ±0.04	7.02 ±0.02
TPU-1	Raw (n=6)	0.76 ± 0.01	2.25 ±0.03	6.99 ±0.02
	Thermoformed (n=6)	0.58 ±0.05	2.21 ±0.04	6.98 ±0.05
TPU-2	Raw	0.76 [24]	n/a	n/a
	Thermoformed (n=6)	0.58 ±0.05	2.23 ±0.03	6.97 ±0.04

Table XIX. Sample dimensions of tested aligner materials by DMA

Material	Conditions	Initial Stress at t=0 h (MPa)	Final Stress at t=2 h (MPa)	Stress Relaxation (%)	Relaxation Rate (MPa/h)
PETG	Thermoformed (n=6)	7.47 ±0.63	2.03 ±0.54	72.89% ±3.01%	2.72 ±0.34
TPU-1	Thermoformed (n=6)	7.85 ± 0.62	1.69 ±0.62	78.52% ±3.41%	3.13 ±0.36
TPU-2	Thermoformed (n=6)	5.21 ±0.38	0.96 ±0.23	81.57% ±3.00%	2.08 ±0.27

Table XX. Creep compliance of raw and thermoformed materials.

Material	Compliance 0 week (1/Pa)	Compliance 1 week (1/Pa)	Compliance 2 weeks (1/Pa)	Compliance 3 weeks (1/Pa)	Compliance 4 weeks (1/Pa)
PETG	$1.66\text{x}10^{-9}$	$1.74\text{x}10^{-7}$	$1.99\text{x}10^{-7}$	$2.14\text{x}10^{-7}$	$2.24\text{x}10^{-7}$
TPU-1	$8.57\text{x}10^{-10}$	$1.51\text{x}10^{-7}$	$1.93\text{x}10^{-7}$	$2.04\text{x}10^{-7}$	$2.14\text{x}10^{-7}$
TPU-2	$9.44\text{x}10^{-9}$	$1.88\text{x}10^{-7}$	$2.51\text{x}10^{-7}$	$2.71\text{x}10^{-7}$	$3.02\text{x}10^{-7}$

Table XXI. Creep modulus of raw and thermoformed materials.

Material	Conditions	Creep Modulus (Pa)
PETG	Raw	2.58×10^{9}
	Thermoformed	6.02×10^{8}
TPU-1	Raw	8.09×10^{9}
	Thermoformed	1.15×10^{9}

Table XXII. Creep compliance of thermoformed materials.

Material	Conditions	Creep Compliance 0 week (1/Pa)
PETG	Raw	3.87×10^{-10}
	Thermoformed	1.66×10^{-9}
TPU-1	Raw	1.24×10^{-10}
	Thermoformed	8.57×10^{-10}

Table XXIII. Creep modulus of thermoformed materials.

Material	Modulus 0 week (Pa)	Modulus 1 week (Pa)	Modulus 2 weeks (Pa)	Modulus 3 weeks (Pa)	Modulus 4 weeks (Pa)
PETG	6.02×10^{8}	5.75×10^{6}	5.02×10^{6}	4.90×10^{6}	4.47×10^{6}
TPU-1	1.15×10^{9}	6.63×10^{6}	5.18×10^{6}	4.68×10^{6}	4.68×10^{6}
TPU-2	1.06×10^{8}	5.31×10^{6}	3.98×10^{6}	3.69×10^{6}	3.31×10^{6}

Table XXIV. Stress relaxation of aligners that were soaked in PBS at different time points

Material	**Testing Conditions**	**Initial Stress at t=0 h (MPa)**	**Final Stress at t=2 h (MPa)**	**Stress Relaxation (%)**	**Relaxation Velocity (MPa/h)**
PETG	Dry (n=6)	7.47 ±0.63	2.03 ±0.54	72.89% ±7.49%	2.72 ±0.34
	24h soaking (n=6)	7.16 ±0.58	1.77 ±0.38	75.28% ±5.51%	2.70 ±0.28
	48h soaking (n=6)	6.87 ± 0.98	1.62 ±0.51	76.41% ±7.23%	2.63 ±0.44
	1wk soaking (n=6)	6.43 ± 0.67	1.46 ±0.37	77.98% ±5.11%	2.59±0.33
TPU-1	Dry (n=6)	7.85 ± 0.62	1.69 ±0.62	78.52% ±3.41%	3.13 ±0.36
	24h soaking (n=6)	7.16 ±0.91	1.30 ±0.77	81.84% ±4.61%	2.93 ±0.40
	48h soaking (n=6)	5.72 ±1.10	0.91 ±0.91	84.09% ±5.12%	2.41 ±0.45
	1wk soaking (n=6)	5.40 ± 0.87	0.69 ±0.76	87.29% ±3.11%	2.36 ±0.25
TPU-2	Dry (n=6)	5.21 ±0.38	0.96 ±0.23	81.57% ±6.43%	2.08 ±0.27
	24h soaking (n=6)	4.79 ±0.41	0.81 ±0.41	83.11% ±4.98%	1.99 ±0.31
	48h soaking (n=6)	4.17 ±0.66	0.64 ±0.55	84.65% ±5.01%	1.77 ±0.38
	1wk soaking (n=6)	4.18 ±0.71	0.59±0.75	85.88% ±4.78%	1.75 ±0.43

Table XXV. Stress relaxation results of TPU-1 aligners in PH 3 Na_2HPO_3/citric acid buffer.

Soak Time (day)	Stress relaxed %	Remaining Stress %
0	78.20 ±2.11	21.80 ± 2.00
1	52.04 ±3.01	47.96 ± 2.98
2	54.77 ±2.56	45.23 ± 2.49
3	54.28 ±1.22	45.72 ± 1.00
7	55.63 ±1.21	44.37 ± 1.18
7 (dried)	56.52 ±2.33	43.48 ± 2.40

.

Table XXVI. Stress relaxation results of TPU-1 aligners in pH 5 Na_2HPO_3/citric acid buffer.

Soak Time (day)	Stress relaxed %	Remaining Stress %
0	78.20 ±2.11	21.80 ± 2.00
1	49.70 ±2.35	49.70 ±2.35
2	52.55 ±2.88	47.45 ±2.56
3	53.28 ± 3.13	46.72 ±3.22
7	54.21 ±3.18	45.79 ± 3.38
7 (dried)	51.34 ±2.11	48.66 ± 2.43

Table XXVII. Creep compliance for three aligners before and after soaking in PBS for 1 week.

Material	Conditions	Compliance (1/Pa)	Normalized % (Soaked/Room Humidity)
PETG	Room Humidity	$1.66\mathrm{x}10^{-9}$	100
	PBS Soaked	$2.38\mathrm{x}10^{-9}$	143
TPU-1	Room Humidity	$8.57\mathrm{x}10^{-10}$	100
	PBS Soaked	$1.43\mathrm{x}10^{-9}$	166
TPU-2	Room Humidity	$9.44\mathrm{x}10^{-9}$	100
	PBS Soaked	$1.37\mathrm{x}10^{-8}$	145

Table XXVIII. Creep modulus for three aligners before and after soaking in PBS for 1 week.

Material	Conditions	Modulus (Pa)	Normalized % (Soaked/Room Humidity)
PETG	Room Humidity	$6.02\mathrm{x}10^{8}$	100
	PBS Soaked	$4.20\mathrm{x}10^{8}$	70
TPU-1	Room Humidity	$1.15\mathrm{x}10^{9}$	100
	PBS Soaked	$6.99\mathrm{x}10^{8}$	61
TPU-2	Room Humidity	$1.06\mathrm{x}10^{8}$	100
	PBS Soaked	$7.30\mathrm{x}10^{7}$	69

Table XXIX. Arrhenius Energy (Ea) of different aligners under dry and soaked conditions.

Aligners	**TPU-1**	**TPU-2**	**PETG**	**TPU-1**	**TPU-2**	**PETG**
Condition	dry	dry	dry	Soaked (1 week)	Soaked (1 week)	Soaked (1 week)
Ea (KJ/mol)	398.21	439.1	388.5	391.1	427.7	373.6
Std (KJ/mol)	5.11	5.50	4.32	4.67	4.62	4.99

VII. REFERENCE

1. Zheng M, Liu R, Ni Z, Yu Z. Efficiency, effectiveness and treatment stability of clear aligners: A systematic review and meta-analysis. Orthod Craniofac Res. Aug 2017;20(3):127-133.

2. Liu CL, Sun WT, Liao W et al. Colour stabilities of three types of orthodontic clear aligners exposed to staining agents. Int J Oral Sci. 12 2016;8(4):246-253.

3. Tindera M. "Out of Silicon Valley, a Billion-Dollar Orthodontics Business Built with Plastic and Patents". Forbes Magazine. 2017 Apr. Available at: "http://www.forbes.com/sites/michelatindera/2017/04/25/out-of-silicon-valley-a-billion-dollar-orthodontics-business-built-with-plastic-and-patents/#10a276ac30c2".

4. ReportsnReports. "Global Invisible Orthodontics Market 2016- 2020.2016". 2016 Sep. Available at:"http://www.reportsnreports.com/Reports/699251-Global-Invisible-Orthodontics-Market 2016-2020.Html".

5. Lombardo L, Arreghini A, Maccarrone R, Bianchi A, Scalia S, Siciliani G. Optical properties of orthodontic aligners-spectrophotometry analysis of three types before and after aging. Prog Orthod. 2015;16:41.

6. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Efficacy of clear aligners in controlling orthodontic tooth movement: a systematic review. Angle Orthod. Sep 2015;85(5):881-9.

7. Elkholy F, Schmidt S, Amirkhani M, Schmidt F, Lapatki BG. Mechanical Characterization of Thermoplastic Aligner Materials: Recommendations for Test Parameter Standardization. J Healthc Eng. 2019;2019:8074827.

8. Iijima M, Kohda N, Kawaguchi K, et al. Effects of temperature changes and stress loading on the mechanical and shape memory properties of thermoplastic materials with different glass transition behaviours and crystal structures. Eur J Orthod. Dec 2015;37(6):665-70.

9. Kravitz ND, Kusnoto B, BeGole E, Obrez A, Agran B. How well does Invisalign work? A prospective clinical study evaluating the efficacy of tooth movement with Invisalign. Am J Orthod Dentofacial Orthop. Jan 2009;135(1):27-35.

10. Kravitz ND, Kusnoto B, Agran B, Viana G. Influence of attachments and interproximal reduction on the accuracy of canine rotation with Invisalign. A prospective clinical study. Angle Orthod. Jul 2008;78(4):682-7.

11. Baldwin DK, King G, Ramsay DS, Huang G, Bollen AM. Activation time and material stiffness of sequential removable orthodontic appliances. Part 3: premolar extraction patients. Am J Orthod Dentofacial Orthop. Jun 2008;133(6):837-45.

12. Djeu G, Shelton C, Maganzini A. Outcome assessment of Invisalign and traditional orthodontic treatment compared with the American Board of Orthodontics objective grading system. Am J Orthod Dentofacial Orthop. Sep 2005;128(3):292-8; discussion 298.

13. Fang D, Li F, Zhang Y, Bai Y, Wu BM. Changes in mechanical properties, surface morphology, structure, and composition of Invisalign material in the oral environment. Am J Orthod Dentofacial Orthop. Jun 2020;157(6):745-753.

14. Zhang N, Bai Y, Ding X, Zhang Y. Preparation and characterization of thermoplastic materials for invisible orthodontics. Dent Mater J. 2011;30(6):954-9.

15. Alexandropoulos A, Jabbari YS, Zinelis S, Eliades T. Chemical and mechanical characteristics of contemporary thermoplastic orthodontic materials. Aust Orthod J. Nov 2015;31(2):165-70.

16. Lombardo L, Martines E, Mazzanti V, Arreghini A, Mollica F, Siciliani G. Stress relaxation properties of four orthodontic aligner materials: A 24-hour in vitro study. Angle Orthod. Jan 2017;87(1):11-18.

17. Min S HC, Yu HS, Lee SB, Cha JY. The effect of thickness and deflection of orthodontic thermoplastic materials on its mechanical properties. Korean J Orthod 2010;40:16-26.

18. Gracco A, Mazzoli A, Favoni O, et al. Short-term chemical and physical changes in invisalign appliances. Aust Orthod J. May 2009;25(1):34-40.

19. Ryu JH, Kwon JS, Jiang HB, Cha JY, Kim KM. Effects of thermoforming on the physical and mechanical properties of thermoplastic materials for transparent orthodontic aligners. Korean J Orthod. Sep 2018;48(5):316-325.

20. Pascual AL, Beeman CS, Hicks EP, Bush HM, Mitchell RJ. The essential work of fracture of thermoplastic orthodontic retainer materials. Angle Orthod. May 2010;80(3):554-61.

21. Kwon JS, Lee YK, Lim BS, Lim YK. Force delivery properties of thermoplastic orthodontic materials. Am J Orthod Dentofacial Orthop. Feb 2008;133(2):228-34.

22. Eliades T, Bourauel C. Intraoral aging of orthodontic materials: the picture we miss and its clinical relevance. Am J Orthod Dentofacial Orthop. Apr 2005;127(4):403-12.

23. Hiromi R YM, Akihiro F, Takashi M, Koutaro M. The mechanical properties of dental thermoplastic materials in a simulated intraoral en- vironment. J Orthod Waves 2006;65:64-72.

24. Ryokawa H MY, Fujishima A, Miyazaki T, Maki K. The mechanical properties of dental thermoplastic materials in a simulated intraoral environment. Orthod Waves. 2006.

25. Ferry JD. Viscoelastic Properties of Polymers. 3rd edition. New York: John Wiley Interscience; 1980.

26. Fang D, Zhang N, Chen H, Bai Y. Dynamic stress relaxation of orthodontic thermoplastic materials in a simulated oral environment. Dent Mater J. 2013;32(6):946-51.

27. Foulc MP BA, Ferry L, Ienny P, Crespy A. Study of hygrothermal ageing of glass fibre reinforced PET composites. Polym Degrad Stabil 2005;89:461–70.

28. Li X, Ren C, Wang Z, Zhao P, Wang H, Bai Y. Changes in force associated with the amount of aligner activation and lingual bodily movement of the maxillary central incisor. Korean J Orthod. Mar 2016;46(2):65-72.

29. Hahn W, Engelke B, Jung K, et al. Initial forces and moments delivered by removable thermoplastic appliances during rotation of an upper central incisor. Angle Orthod. Mar 2010;80(2):239-46.

30. Ercoli F, Tepedino M, Parziale V, Luzi C. A comparative study of two different clear aligner systems. Prog Orthod. May 2014;15(1):31.

31. Pithon MM. A modified thermoplastic retainer. Prog Orthod. Sep 2012;13(2):195-9.

32. Yang B, Li C and Chor JH. Effects of moisture on the glass transition temperature of polyurethane shape memory polymer filled with nano-carbon powder. European Polymer Journal. 2005. 41.1123– 1128.

33. Finnie S, Prasad KV, Sheen DB, Sherwood JN. Microhardness and dislocation identification studies on paracetamol single crystals. Pharm Res. May 2001;18(5):674-81.

34. Boubakri A, Guermazi N and Ayedi HF. Investigations on hygrothermal aging of thermoplastic polyurethane material. Materials & Design. 2009.Vol 30. pp 3958-3965.

35. Instron Model Manual. Available at: http://fab.cba.mit.edu/content/tools/instron/M10-94400-1.pdf.

36. ISO. Dentistry Base Polymers: Part 2: Orthodontic Base Polymers, ISO 20795-2, Geneva, Switzerland, 2013.

37. Elkholy F, Jager F and Lapatki BG,. Forces and moments applied during derotation of a maxillary central incisor with thinner aligners: an in-vitro study. Am J Orthod Dentofacial Ortho.Vol. 151, No. 2, pp. 407–415, 2017.

38. Nielsen LE. Mechanical properties of polymers and composites (Onogi S). 1st ed. Kyoto: Kagakudojin; 1976.

39. Kikutani T IH. Analysis of crystalline orientation by wide-angle X-ray diffraction. J Jpn Soc Polym Process 2000;12:556–60.

40. Hahn W, Dathe H, Fialka-Fricke J, et al. Influence of thermoplastic appliance thickness on the magnitude of force delivered to a maxillary central incisor during tipping. Am J Orthod Dentofacial Orthop. Jul 2009;136(1):12.e1-7; discussion 12-3.

41. Fernandez J, Larranaga A, Etxeberria A, Sarasua JR. Tensile behavior and dynamic mechanical analysis of novel poly(lactide/δ-valerolactone) statistical copolymers. J Mech Behav Biomed Mater. 2014 Jul.

42. Schuster S, Eliades G, Zinelis S, Eliades T, Bradley TG. Structural conformation and leaching from in vitro aged and retrieved Invisalign appliances. Am J Orthod Dentofacial Orthop. Dec 2004;126(6):725-8.

43. Huget EF, Patrick KS, Nunez LJ. Observations on the elastic behavior of a synthetic orthodontic elastomer.J Dent Res. Feb 1990;69(2):496-501.

44. Alwis NC and Burgoyne CJ. Time-temperature superposition to determine the stress rupture of aramid fibres. Appl. Compos. Mater; 2006. p. 249–264.

45. Boubakri A HN, Elleuch K, Bienvenu Y. Impact of aging conditions on mechanical properties of thermoplastic polyurethane. Mater Des 2010;31:4194-201

46. Airoldi G, Riva G, Vanelli M, Filippi V, Garattini G. Oral environment temperature changes induced by cold/hot liquid intake. Am J Orthod Dentofacial Orthop. Jul 1997;112(1):58-63.

47. Ye Y, Di P, Jia S, Lin Y.Occlusal force and its distribution in the position of maximum intercuspation in individual normal occlusion: a cross-sectional study. Zhonghua Kou Qiang Yi Xue Za Zhi. Sep 2015;50(9):536-9.

48. Kattan M, Dargent E, Ledru J and Grenet J. Strain-induced crystallization in uniaxially drawn PETG plates. J Appl Polym Sci; 2001.

49. Bradley T, Eliades G, Zinelis S and Eliades T. Do the mechanical and chemical properties of Invisalign TM appliances change after use? A retrieval analysis. Eur J Orthod 2016;38: 27-31.

50. Azhikannickal E and Zak G. Laser light transmission through thermoplastics as a function of thickness and laser incidence angle: experimental and modeling. J Manuf Sci Eng 2012;134:061007.

51. Baumgartner JN, Yang CZ, Cooper SL. Physical property analysis and bacterial adhesion on a series of phosphonated polyurethanes.Biomaterials. Jun 1997;18(12):831-7.

52. Valentova H NJ, Ilavsky M and Pissis P. Dynamic mechanical and water sorption behaviour of ordered polyurethanes. J Non-Cryst Solids 2002;307– 310:304–10.

53. H L, Y. H. Preparation of Water-Absorbing Polyacrylonitrile Nanofibrous Membrane. Macromol Rapid Commun. 2006. pp. 142-145.

54. Li L, Xiang T, Tang M, Zhao W, Sun S and Zhao C. Modification of polyethersulfone hemodialysis membrane by blending citric acid grafted polyurethane and its anticoagulant activity. Journal of Membrane Science. 2012. Vol 405–406.pp. 261-274.

55. Andrade JD. Surface Interfacial Aspects of Biomedical Polymers. Vol. 1. Plenum Press, New York. 1985. pp. 249.

56. Wu S. Polymer Interphase and Adhesion.Marcel Dekker. New York/Basel.1982.

57. Hinrichsen G. Polyurethane Handbook. Munich: Hanser Publishers. 1994.

58. Mamunya YP SV, Lebedev EV, Pissis P, Kanapitsas A, Boiteux G. Structure and water sorption of polyurethane nanocomposites based on organic and inorganic components. Eur Polym J. 2004;40:2323–31.

59. Jasso-Gastinel C. Modification of Polymer Properties. 1st ed. 2017.

60. Gopferich A. Mechanisms of polymer degradation and erosion. Biomaterials 1996;17:103-14.

61. Oprea S VS. Evaluation of physico-mechanical properties of precipitated polyurethane films in medium of free radical agents. Eur Polym J 2002;38: 1465–70.

62. Baschek G HGaZF. Effect of water absorption in polymers at low and high temperatures. Polymer.1999. 40, 3433–3441.

63. Béhin P SG, Ruse ND. and Sadoun M. Dynamic mechanical analysis of high pressure polymerized urethane dimethacrylate. Dental Materials.2014. 30, 728–734.

64. Gonçalves ES PL and Ogilby PR. Mechanism of the temperature-dependent degradation of polyamide 66 films exposed to water. Polym Degrad Stabil 2007;92:1977–85.

65. Ahn HW and Kim SH. A new type of clear orthodontic retainer incorporating multi-layer hybrid materials. Korean J Orthod 2015;45:268-72.

66. Hollande S LJ. Weight loss during different weathering tests of industrial thermoplastic elastomer polyurethane-coated fabrics. Polym Degrad Stabil. 1998; 62: 501-505.

67. Bramhecha I SJ. Development of sustainable citric acid-based polyol to synthesize waterborne polyurethane for antibacterial and breathable waterproof coating of cotton fabric. Industrial & Engineering Chemistry Research. 58 (47), 21252-21261.

68. Schaefer F WH. Stabilized plasticizers for thermoplastic polyurethane. Available at" https://patents.google.com/patent/WO2015113874A1/en".

69. Zanni-Deffarges MP SM. Diffusion of water into an epoxy adhesive: comparison between bulk behaviour and adhesive joints. Int J Adhes Adhes 1995;15:137–42.

70. Synge JL. The theory of an incompressible periodontal membrane. Int J Orthod Dent Child 1933;19:567-73.

71. Natali A PP, Carniel E and Dorow C. Viscoelastic response of the periodontal ligament: an experimental-numerical analysis. Connect Tissue Res 2008;45:222-30.