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Effects of Simulated Functional Loading Conditions on Dentin, Composite, and Laminate Structures

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Abstract

Use of composite restorations continues to increase, tempered by more potential problems when placed in posterior dentition. Thus, it is essential to understand how these materials function under stress-bearing clinical conditions. Since mastication is difficult to replicate in the laboratory, cyclic loading is frequently used within in vitro evaluations but often employs traditional fatigue testing, which typically does not simulate occlusal loading because higher stresses and loading frequencies are used, so failure mechanisms may be different. The present investigation utilized relevant parameters (specimen size; loading frequency) to assess the effects of cyclic loading on flexural mechanical properties and fracture morphology of (coronal) dentin, composite, and dentin-adhesive-composite “laminate” structures.

Incremental monitoring of flexural modulus on individual beams over 60,000 loading cycles revealed a gradual increase across materials; post-hoc comparisons indicated statistical significance only for 1 versus 60k cycles. Paired specimens were tested (one exposed to 60k loading cycles, one to static loading only), and comparisons of flexural modulus and strength showed statistically significantly higher values for cyclically-loaded specimens across materials, with no observable differences in fracture morphology. Localized reorganization of dentin collagen and polymer chains could have increased flexural modulus and strength during cyclic loading, which may have implications toward the life and failure mechanisms of clinical restorations and underlying tooth structure.

Keywords

cyclic loading; dentin; laminate; restoration failure; flexure

INTRODUCTION

With improvements in dental composites, there has been a substantial increase in the use of composite restorations in the posterior dentition as an alternative to dental amalgam.^{1,2}

However, the frequency of restoration failure is higher for posterior composite as compared to either posterior amalgam or anterior composite restorations.^{2,3} In order to understand this higher failure rate, it is important to consider the conditions to which a restoration is exposed. These include a harsh oral environment⁴ and cyclic loading associated with mastication⁵⁻⁷. Although the entire dentition is exposed to this oral environment, the occlusal stress is much higher in the posterior segment as compared to the anterior segment^{8,9}, which might be linked to the increased failure of posterior composites.

It is very difficult to replicate mastication in the laboratory⁷ due to masticatory stress variations between individual teeth and varying intervals of tooth-tooth contact during the chewing cycle^{5,7}. As a result, there is an extensive body of work studying fracture by static/quasistatic loading only.¹⁰⁻¹³ The majority of cyclic loading studies that have been performed on dentin¹⁴⁻¹⁶ and composite¹⁷⁻²⁰ are traditional fatigue studies using higher stresses and frequencies than are associated with mastication²¹, and thus, do not simulate occlusal loading conditions and may not reflect clinical function failure mechanisms.

Because fatigue testing takes a significant amount of time to perform awaiting failure of a specimen^{14,19}, it is not necessarily practical for testing human teeth and associated dental restorative materials. Cyclic loading with clinically-relevant testing parameters (aqueous/electrolyte environment associated with saliva, intra-oral temperature, and loading conditions) and using a finite number of loading cycles might be a more feasible option for testing dental materials, to more closely replicate clinical function. In order to thoroughly understand restoration failure, it is important to study the three associated components: the restorative material, coronal dentin into which restorations are typically placed, and the dentin-adhesive-composite laminate structure developed when a tooth is restored. Available information on the effects of cyclic loading on human coronal dentin^{15,22} and laminates^{23,24} comes from fatigue testing methods, and to date, there is only one study in which composite specimens sized on the order of a single-tooth restoration, rather than the ISO standard²⁵ of $25 \times 2 \times 2$ mm beams, have been tested²⁶.

Therefore, the purpose of this study was to investigate the effects of cyclic loading on coronal dentin, dental composite, and laminate structure to determine changes in flexural properties that might lead to posterior composite restoration failure. The intent of cyclic loading was not to test to failure, but to expose the specimens to a finite number of loading cycles using clinically pertinent parameters (specimen size, testing mode, loading rate, and stress levels) to more closely simulate clinical function. In addition, scanning electron microscopy (SEM) was used to characterize the morphology of the fracture sites. Four hypotheses were tested within this investigation: that there was an incremental effect of cyclic loading on flexural modulus of material (dentin, composite, laminate), and that there was a differential effect of loading (cyclic versus static) on flexural modulus, flexural strength, and fracture morphology of each material.

MATERIALS AND METHODS

Determination of Study Parameters

Prior to specimen preparation and mechanical testing, it was necessary to determine the parameters to be utilized in this study: specimen storage and testing solution, testing mode, stress levels, loading rate, and specimen size. Choice of specimen storage solution is very important, in order to relate findings from in vitro studies to clinical conditions. Distilled water is a typical storage solution²⁷⁻³⁰, but has been reported to affect both the mechanical properties²⁹ and spectroscopic profile²⁸ of human dentin. Phosphate buffered saline (PBS) with its electrolyte composition is more similar to saliva than distilled water⁴, and despite slight spectroscopic changes reported for dentin stored in a PBS solution²⁸, mechanical

properties were not shown to be significantly affected in the short term, up to 15 days^{27,29}. Thus, PBS used over short periods should more closely represent the oral aqueous environment and was therefore chosen for specimen storage in this study.

The coronal region of dentin specifically deserves investigation because it is where restorations are typically placed. Three-point bending is a flexure mode that is both clinically relevant (class I and II posterior composites, for example)³¹ and used in ISO specification testing for dental restoratives²⁵. Because of higher posterior composite failure rate and higher occlusal stresses in the posterior segment, it was necessary to utilize stress values associated with molar occlusion. An in-depth literature search of studies on the loading of the dentition during mastication of various foods^{6,32-35} revealed that a value of 10.5MPa is consistent with the occlusal stress on a molar (in many cases, conversion using occlusal contact areas³⁶ was necessary to normalize the values for comparison). Data were considered from the final or occlusal stage of mastication, where teeth are in intimate contact.⁵ Studies using food rather than a non-edible device were deemed more critical to the current investigation to allow for the tooth-tooth contact of the final stage of mastication. It was also important to simulate the loading rate associated with mastication, which has been reported to range between 0.5-3 Hz^{7,37}, so a value of 2Hz was chosen. Use of these parameters (10.5MPa and 2Hz) allows a baseline to be established and a simplified model to be tested. The authors are aware that during mastication, there may be periods of increased stress due to different foods, patient-to-patient variability, or abnormal chewing behaviors, and these may result in a more complex stress state, but these variables and the highest stresses developed during mastication were not the focus of the present study.

On average, teeth undergo approximately 10^6 chewing cycles annually.⁷ The purpose of this study was not to determine the failure point, but to expose specimens to a finite number of loading cycles to observe changes in mechanical properties. In a previous cyclic loading study³⁸, flexural modulus differences in resin composite cement (similar in chemistry to dental composite³⁹) was reported as a result of 15k loading cycles. A study of human dentin using traditional fatigue testing²² reported an increase in flexural modulus over 0-15k loading cycles. Because this was the first study to incrementally analyze when flexural properties might be changing as a function of loading cycles simulating mastication rate and stress, measurements were made at 1, 1k, 5k, 15k, 35k, and 60k. Although 60k cycles represents approximately 25 days of chewing (2400 daily tooth contacts⁴⁰⁻⁴²), a goal of the study was trying to understand when property changes begin. Moreover, there were limitations related to instrumentation over time, since the loading rate was kept at chewing rate, 2Hz. It was also decided that beam specimens would be from the mesio-distal orientation of a human third molar, and dimensions would be consistent with the size of a beam from this orientation ($6 \times 1 \times 1$ mm, with a tolerance of ± 0.2 mm for each dimension), as this would relate to restoration placement location and size.

This combination of study factors was chosen to at least minimally simulate posterior dentition intra-oral conditions. A power analysis based on preliminary data determined that ten specimens each of dentin, composite, and laminate specimens per condition (exposed to cyclic loading versus static loading only) would detect a meaningful flexural property change of 25% at $\alpha=0.05$ and power $1-\beta=0.80$. This meant that a total of 60 specimens (30 cyclic and 30 static) would be tested.

Specimen Preparation

Institutional Review Board (IRB) approval (Study #05-27e) was obtained in order to collect unerupted third molars (with no patient identifiers) already scheduled for extraction. Following extraction, teeth were stored at 4°C in Dulbecco's PBS (Sigma-Aldrich, St. Louis, MO) with 0.002% sodium azide (pH = 7.0) to inhibit bacterial and fungal

contamination. Teeth were visually investigated for obvious defects and were tested within one month of extraction to assure no changes in mechanical properties due to storage time.

For dentin specimens, the occlusal one-third of the crown was removed and the cut dentin surface was viewed under a light microscope (Nikon Instruments, Inc., Melville, NY) at 200X magnification to ensure that no enamel remained. This region was utilized because the tubules are oriented in relatively the same direction throughout the thickness and a reasonable dentin bonding surface (without enamel) can be achieved.⁴³ Beams were sectioned from the middle of the cut dentin surface using a low-speed, water-cooled diamond saw (Buehler, Ltd., Lake Bluff, IL). Two beams were sectioned from each tooth, with one beam assigned to either cyclic or static loading conditions. After sectioning, beams were again viewed at 200X to make certain no obvious defects were present precluding specimen use for testing.

Z250 composite (3M-ESPE, St. Paul, MN; lot numbers 5PH, 6CT, and 6CU) was selected based on wide clinical use as a posterior restorative material. To prepare composite beams, two glass microscope slides were used as a template. Under ambient laboratory conditions with yellow light, Z250 composite was injected onto the surface of one microscope slide in the shape of a disc (approximately 20 mm radius $\times$ 2 mm thickness). Next, the second microscope slide was placed atop the disc and the two slides were gently pressed together to a uniform thickness of 1-1.5mm, controlled by glass “stops” (which limited the amount of compression with the upper slide) attached to either end of the bottom slide. To remove any bubbles or voids present, the template was held on a vibrating table (Buffalo Dental Manufacturing Company, Syosset, NY) for 15s per side. Following the manufacturer’s instructions, specimens were polymerized (Elipar TriLight; ESPE, Seefeld, Germany) for 20s intervals across each surface of the disc (top and bottom), with 9 irradiation events per side (sites are presented in Figure 1) to ensure that the entire surface was exposed to the light. Light output as measured with the integrated radiometer was approximately 500mW/cm². Following removal of the microscope slides, the resultant composite disc was sectioned into two beams (one for cyclic loading, one for static loading). Although individual composite beams could have been fabricated eliminating the need for sectioning, because dentin and laminate specimens had to be sectioned, composite beams were fabricated in such a way as to have a similar sectioning protocol. This provided consistency between the various specimen types and would potentially account for any effect sectioning might have. Since clinical placement of composite is done incrementally to optimize polymerization and this was not possible with the disc fabrication, sectioned beams were polymerized again using two 20s intervals across both the newly formed (sectioned) surfaces (represented by letters A-D in Figure 1), as a means of enhancing polymerization. As with dentin, the composite beams were viewed with a light microscope to make certain no obvious defects were present.

Laminate beams consisted of an adhesive layer sandwiched between dentin and composite (Z250), as would exist in a restored tooth. Following the removal of the occlusal one-third as described for dentin, the sectioned tooth surface was polished manually using wet 320 grit sandpaper for 30s to produce a smear layer. The prepared tooth surface was then treated with Scotchbond Multi-Purpose adhesive (3M-ESPE), a three-bottle system (etchant, lot number 5EX; primer, lot number 5AW; and adhesive, lot number 5PB), according to the manufacturer’s instructions. Following adhesive polymerization for 10s, Z250 was condensed onto the prepared tooth surface to yield a composite thickness of approximately 1.5-2mm. A glass slide gently pressed onto the composite produced a flattened surface and a uniform composite thickness of approximately 1-1.5mm. The resulting disc shape was slightly smaller in diameter than the tooth itself, to assist in locating the interface between the dentin and composite during the beam sectioning step. When the composite was

polymerized through the glass slide, only 4 irradiation events were required (equaling 80s total polymerization). Two beams (one for cyclic, one for static) were cut such that one-half of the specimen was mid-coronal dentin and one-half was composite, with the dentin-adhesive interface centered in between. After sectioning, beams were polymerized again using two 20s intervals along both newly formed surfaces (an additional 80s), as described for composite beams (Figure 1). As with composite and dentin, beams were checked with the microscope to make certain no obvious defects were present and to confirm approximately equivalent thickness of dentin and composite layers; presence of defects or unequal thicknesses prevented use of the specimen pair. A schematic diagram of specimen origin and design is presented in Figure 2.

Mechanical Testing

Prior to mechanical testing, individual beams of each material (dentin, composite, and laminate) were stored within individual glass vials (Fisherbrand, Fisher Scientific, Pittsburgh, PA) containing 10 mL of PBS for 24 hours at 37°C. To have equivalent PBS storage time for both the cyclic and static specimens, static specimens were stored the additional length of time required to expose specimens to 60k loading cycles (approximately 8.5 hours), thus exposing all specimens to PBS for a total of 32.5 hours, instead of the 24 hours described in ISO 4049:2000(E).25

All mechanical testing was performed with the EnduraTEC ELF 3300 (Bose Corporation, Eden Prairie, MN) using a three-point bend fixture (Figure 3) with two rods (diameter = 2mm) mounted parallel with 5 mm between centers (support span = 5mm). Specimens were immersed in 37±1°C circulating PBS throughout testing. Dentin beams were oriented with superficial dentin as the top surface. Laminate specimens were always loaded so the composite was the top surface. Specimens were either (1) statically loaded to failure (n=10 per material) at 0.5mm/min or (2) cyclically loaded (n=10 per material) with stress values varying between approximately 4MPa (enough to maintain a slight load on the specimen) and 10.5MPa (consistent with molar occlusion) over 60k loading cycles, followed by testing to failure by static load at 0.5mm/min (the same loading rate as for specimens exposed to static loading only).

Flexural Property Measurements

The flexural modulus and strength were calculated from load-displacement diagrams generated during cyclic and static loading. Flexural modulus was calculated from the straight-line portion as follows:

$$E_{\text{flexural}} = \frac{L^3 m}{4bd^3} \quad (1)$$

where E_{flexural} = elastic modulus in bending (flexural modulus, GPa) with L = support span (mm), m = slope of the straight-line portion of the stress-strain diagram (N/mm), b = width of beam (mm), and d = depth of beam (mm)²⁵. With cyclic specimens, modulus measurements were following 1, 1k, 5k, 15k, 35k, and 60k cycles. For the static specimens, one modulus measurement was made when specimens were tested to failure. For the cyclic specimens, with multiple modulus measurements made on the same specimen, rather than testing to failure, specimens were stressed to ~25 MPa by loading the specimen to approximately 5N following the indicated number of cycles and the modulus calculated from the associated load-displacement diagram. To provide consistent comparisons, modulus measurements for all specimens (cyclic and static) were made from a similar portion of the load-displacement diagrams. Figure 4 includes representative load-

displacement curves for a static and a 60k-cyclic composite specimen. As already described and indicated by the arrow in the load-displacement diagram, the modulus was measured from the initial aspect of the curves between 0-5 N load.

Flexural strength was calculated using the following equation:

$$\sigma_{\text{flexural}} = \frac{3PL}{2bd^2} \quad (2)$$

where σ_{flexural} = stress (MPa) in the outermost layers at the midpoint and P = load (N) at fracture (other variables identical to equation 1). Because flexural strength is calculated based on maximum load to failure, only one flexural strength calculation was performed for each static and cyclic specimen (after 60k cycles).

SEM Fractographic Analysis

Four representative specimens collected from each type of loading and each type of material were mounted on aluminum SEM stubs using colloidal graphite, then sputter coated with gold/palladium for approximately 30s and imaged in secondary electron mode at 15.0kV (XL30 field-emission SEM; Philips Electron Optics, Hillsboro, OR). All specimens were imaged at low magnification (50X) at 25° tilt to better display crack front. Dentin and composite specimens were further imaged at 1000X and 5000X. The three different major regions of the laminates (dentin, adhesive, and composite), were imaged separately, dentin and composite as described above, and adhesive at 1000X only.

Statistical Analysis

To determine if there was an incremental effect of cyclic loading on flexural modulus of material (dentin, composite, laminate), flexural modulus values calculated at 1, 1k, 5k, 15k, 35k, and 60k cycles on the same specimen were compared using a Repeated Measures Analysis of Variance (RM-ANOVA) at $\alpha=0.05$ with significant differences analyzed using the Fisher-Hayter post hoc test. To determine if there was a differential effect of loading (cyclic versus static) on flexural modulus or flexural strength of material (dentin, composite, laminate), comparisons were made between the paired specimens (one exposed to cyclic loading (60k loading cycles), one to static loading only) via RM-ANOVA at $\alpha=0.05$. Presence of qualitative differences in fracture morphology was determined by comparing representative SEM images of paired specimens. For dentin specimens, it was important to determine if the crack had obviously changed planes during the course of propagation. In composite specimens, comparisons of specimen surface morphology and associated secondary cracking relative to the matrix and filler were most critical. With laminate specimens, it was vital to see if there were differences at the dentin-adhesive interface and if the individual component materials behaved differently at failure when adhered together as in a restored tooth.

RESULTS

Flexural Mechanical Properties

Mean flexural modulus and standard deviation (SD) measurements after 1, 1k, 5k, 15k, 35k, and 60k loading cycles across materials are presented in Table 1. The flexural modulus value of dentin is slightly higher after each loading increment than the values for composite or laminate, and laminate values appear to be between dentin and composite for nearly all loading increments. However, the trend of increasing flexural modulus remains the same for all three materials across 60k loading cycles, and the means across cycles are similar for

each material. Although there was no statistically significant interaction effect ($p>0.05$) between material and loading cycles, there was a significant increase ($p\leq 0.05$) in modulus as a function of loading cycles. However, the Fisher-Hayter post hoc test indicated the modulus increase was significant ($p\leq 0.05$) only between modulus measured after 1 cycle as compared to the modulus after 60k cycles across materials. Therefore, the hypothesis that loading cycles caused a significant effect on incremental flexural modulus measurements across materials was accepted.

Paired specimens were used to determine if there was a differential effect of loading (cyclic versus static) on flexural modulus or flexural strength of material (dentin, composite, laminate). These specimens originated in the same tooth (in the case of dentin and laminate) or composite disc. The results of this testing (mean flexural modulus and flexural strength and SD) are presented in Table 2. The data show an increase in both flexural modulus and strength as a function of cyclic loading. With flexural modulus, there was a nearly two-fold increase after cyclic loading for all materials, while the relative increase in flexural strength after cyclic loading was much smaller. The results of the separate two-way RM-ANOVAs indicated there was no statistically significant interaction effect between material and loading ($p>0.05$) but the main effect of loading was statistically significant ($p\leq 0.05$). Thus, the hypotheses that there was a significant difference in flexural modulus or flexural strength across materials as a function of loading (cyclic versus static) were accepted.

SEM Fractographic Analysis

To determine if there was a differential effect of loading (cyclic versus static) on the fracture morphology of dentin, composite, and laminate, as determined by SEM, representative images of the fracture surfaces of specimens of each material from the tested paired specimens were used for the fractographic analysis.

Representative images of cyclic and static loaded dentin beams appear in Figure 5. An inverted “lip” is apparent on the compressive (upper) beam surfaces with subtle lines radiating from the tensile surface (5a, d) suggesting the crack origin. The cyclic loaded specimens display evidence of the crack changing planes, which creates a “step” between the planes of tubules (5c). Cracking has cross-sectioned tubules horizontally in some regions and vertically in others. Similar features exist for the static loaded specimens.

Representative images of cyclic and static loaded composite beams appear in Figure 6. The tensile region (lower surface) of both the cyclic and static specimens exhibit subtle lines radiating outward from a defect in the lower left-hand portion of each figure; these lines are less apparent at higher magnification (6b, c, e, f). Crack propagation appears to be within the resin matrix, as the filler particles are covered with resin in either cyclic (6c) or static specimens (6f). Similar to the dentin images, a lip is visible on the compressive (upper) beam surface (6a, d).

The three different regions of representative laminates (dentin, adhesive, and composite) were imaged separately and presented in Figure 7. Although the dentin region was always on the tensile side of bending (lower surface), which is where the origin of cracking would be expected, there is no obvious origin in the cyclic or static specimens (7a, g). The cyclic and static fractured laminate dentin regions appear similar to dentin beam fracture surfaces, with minor differences, including a larger step in the dentin (nearest the laminate) (7b, c, h, i). Similar to dentin and composite specimen, there was a lip within the compressive region (containing the composite) of both the cyclic (7e, f) and static laminate (7k, l). Again similar to composite specimen, crack propagation tended to be through the resin matrix in both cyclic (7d) and static (7j) specimens. With the adhesive layer being located at the neutral

zone of bending, it is not surprising that adhesion was well maintained between the composite and dentin sections of the laminate.

In general, fractographic analysis of dentin, composite and the laminate specimens did not suggest any differences between type of loading within each material. Therefore, the hypothesis that there was a differential effect of loading (cyclic versus static) on the qualitative fracture morphology across materials was not substantiated by the results.

DISCUSSION

In the current investigation, it was critical to replicate clinical function as closely as possible within the limitations of in vitro testing. Cyclic loading levels and frequency, specimen size, and environmental conditions were all chosen to simulate oral conditions, in order to assess the effects of cyclic loading on dentin, composite, and laminate flexural modulus and strength and fracture morphology.

It has only been within the past five years that human dentin has been investigated under dynamic conditions. Within the current study, both dentin modulus and strength increased as a result of 60k loading cycles. To date, there has been only one previous study²² in which dentin was studied under dynamic conditions and the flexural modulus was also reported to increase. In that study, increasing flexural modulus was observed over 0-15k loading cycles in dentin from patients 25±5 years of age and was attributed to the viscoelastic nature of dentin collagen and viscoelasticity effects caused by a non-zero mean stress, as well as collagen potential realignment resulting from tensile stress.⁴⁴ Another study by Carden and colleagues⁴⁵ reported collagen structural changes in bone as a function of deformation, using micro-Raman spectroscopy. The results of both those previous studies might help explain the incremental increase in dentin flexural modulus over 1 to 60k loading cycles leading to the modulus difference between the paired static and cyclic specimens. However, it has also been reported that the collagen realignment may be reversible⁴⁴, but this has not been confirmed. Previous studies, including the current study, have not allowed enough relaxation time for this to occur. Therefore, a future study incorporating a short waiting period after the final cyclic loading would be necessary to confirm the reversibility of the localized collagen reorganization.

Comparison of dentin static flexural modulus between the present study and the study by Arola and Replogel¹⁵ indicates that the modulus in their study was approximately 2 times higher than in the current study. In that study, modulus measurements were made on load-displacement curves generated when specimens were tested to failure. In this study, in order to make multiple modulus measurements of the same cyclic specimen across the various cycle intervals, the specimens were not tested to failure but instead, the modulus was based on load-displacement curves generated when specimens were stressed to approximately 25 MPa. For consistent modulus comparisons within this study, all modulus measures (cyclic and static) were made from a similar portion of the load-displacement curve. However, this would likely result in a lower modulus than if the entire linear aspect of a static failure curve was considered in the calculation and accordingly is a limitation of the current study. Moreover, there were a number of other differences between the current study and Arola and Replogel's, 1:1 vs 0.5: 1.5 ratio between specimen height and width; 3-point vs 4-point bending, and 0.25 vs 0.1 stress ratios, respectively. Arola and Replogel¹⁵ were also able to precisely identify the age of the patients whose teeth were used, a source of information not possible in the present study due to privacy limitations and availability of teeth. Collectively, these factors might have contributed to the differences in modulus values.

In the current study, dentin flexural strength, as with flexural modulus, also increased with cyclic loading. In contrast to the current results, previous dentin fatigue studies^{15,22} have reported that dentin flexural strength decreases under fatigue testing conditions. However, the testing protocols are very different in the current investigation as compared to a fatigue study with stresses at least five times higher^{15,22} and loading frequencies greater than those of mastication. Unfortunately, to date, there is no other reported study using cyclic stress values simulating masticatory function with a finite number of loading cycles to measure changes in dentin flexural properties, so there is no comparison data. In the current study, the low testing frequency rate (2 Hz) may have resulted in collagen chain re-arrangement⁴⁴, as described previously for dentin flexural modulus, that could also potentially explain the increase in flexural strength. Dentin toughening has also been reported with crack length increases.¹³ Collectively, toughening during cracking plus the incomplete relaxation of potentially realigned collagen within the dentin might play a fundamental role in the increase in flexural strength after 60k loading cycles.

The increase in flexural modulus for composite in the current study was not unanticipated, because such an increase has been reported in the polymer literature.⁴⁶⁻⁴⁸ The increase in modulus in the amorphous regions of polyethylene has been attributed to realignment of the polymer chains along the extension axis at low extension rates.⁴⁶ Loading-induced crystallization or localized order is also possible in some polymer systems⁴⁹, and crystallites can function as cross-links⁴⁷, thus restricting molecular motion, at least while present, as they may be temporary⁵⁰. Within the dental literature, another study which utilized parameters similar to this study to test resin composite cement³⁸ also reported an increase in flexural modulus after 13k loading cycles and attributed this increase to reorganization within the resin matrix. Z250, the composite used in the present study, has a highly cross-linked amorphous matrix similar to the structure of resin composite cement.³⁹ Although cross-linking will significantly reduce the mobility of the chains, there is the potential for realignment within localized regions, similar to constraint of collagen between dentinal tubules. It is also important to note that the Z250 static flexural modulus values in the current study are lower than what has been previously reported; however, just as with dentin, those differences are probably related where the modulus measurements were made on the load-displacement diagrams.

The increase in composite flexural strength following cyclic loading can also be related to previous polymer literature. A slight increase in tensile strength has been reported to occur with uniaxial extension of polyethylene⁴⁶ and in natural and synthetic rubbers⁵⁰. The increase in strength, as with modulus, has been attributed to chain realignment along the extension axis at low extension rates in the amorphous regions of polyethylene⁴⁶ and crystallization in natural and synthetic rubbers⁵⁰. Not surprisingly, just as with the dentin fatigue studies, the results of the current study are not supported by previous composite fatigue studies, which report a decreased fatigue life with increasing loading cycles and indirectly, decreased strength.^{19,20} However, higher loading rates and stress levels than are associated with mastication were used in those studies. Many studies have reported a fatigue limit for composite materials; specifically, a value of 45.9MPa has been cited for $25 \times 2 \times 2$ mm beams of Z250.²⁰ Clearly, this is well above the maximum cyclic stress applied in the current study.

In developing the laminate to simulate a restored tooth, the specimen contained dentin (inferior half) and composite (superior half) with a hybrid layer of demineralized dentin collagen encased by adhesive resin providing a micromechanical bond between the dentin and composite.⁵¹ The laminate specimens, as with dentin and composite, exhibited a gradual increase in flexural modulus over 60k loading cycles leading to a difference in modulus between the paired cyclic and static specimens. Similarly, the flexural strength of

the laminate specimens also increased in the cyclic specimens. The same explanations of increased modulus and strength for dentin and composite would appear to apply to the laminates, since these two materials are the major components.

In order to confirm whether potential reorganization and realignment of dentin collagen fibrils and the composite resin matrix are associated with the flexural properties changes exhibited in the cyclically-loaded dentin, composite and laminate specimens, future studies would be required. Instrumentation, such as micro-Raman spectroscopy, could potentially be an effective non-destructive analysis that could be used to study the process in situ.

SEM fracture analysis was included in this investigation to provide a micromechanistic link between potential flexural property changes and associated fracture/failure mechanisms in dentin, composite or laminate as a function of different loading conditions. In the current study, there were no observable differences in fracture morphology between cyclic and static specimens, thus the fractographic analysis did provide any explanation for the increased flexural mechanical properties following cyclic loading.

As with any in vitro study, there are always limitations. For example, the beams in the current study were loaded to only 60k cycles; this represents a very short time of function considering that most teeth are subjected to close to a million loading cycles per year.⁷ With the low stress level and only 60k cycles, the current investigation protocol may not have produced enough damage to decrease strength. A future study incorporating more long-term cyclic loading and potentially including some rest periods would more closely simulate function across time.

Another potential study limitation is that with the current laminate model, neither the dentin nor composite would be as prone to flexure as the beam specimens. In a restored tooth, both dentin and composite flexural property changes as a function of cyclic loading might not be as problematic, depending on the type of restoration. For example, a mesio-occlusal-distal (MOD) restoration (Class II) would be more prone to flexure than a very small Class I occlusal restoration that has only one unbonded surface. Besides the potential differences in flexure between Class I and II restorations, there are distinct differences in the restoration margins. With a Class I, all margins are composite-enamel interfaces; in contrast, the Class II has mesial and/or distal gingival margins with minimal to no enamel resulting in a composite-dentin marginal interface. At these sites, changes in flexural properties over time, especially with the MOD restoration being more prone to flexure than the associated dentin, could possibly lead to marginal interfacial problems. Potentially, this could help explain why there are much higher failure rates associated with the mesial and distal gingival margins of Class II restorations.³ To confirm this, a future study using cyclic loading of restored teeth would be required.

One of the goals of this study was to use a simple model to simulate physiologic conditions associated with a posterior composite restoration and provide insight into potential changes in the flexural properties of dentin, composite, or dentin restored with composite (laminate) that might lead to posterior composite restoration failure. Overall, the flexural property data indicate that dentin, composite, and laminate become stiffer and stronger with cyclic loading up to 60k loading cycles. The implication is that a composite restoration and the associated interfacial structure would experience mechanical property changes over time with functional loading, which could conceivably be detrimental to the composite-dentin bond and contribute to restoration interfacial failure.

CONCLUSIONS

The following conclusions can be drawn, within the limitations of this in vitro investigation on the effects of cyclic loading on dentin, composite, and laminate:

1. Irrespective of material, there was an increase in flexural modulus across loading cycles, but the increase was significantly higher ($p \leq 0.05$) only after 60k loading cycles as compared to 1 cycle.
2. Irrespective of material, cyclic flexural modulus was significantly higher ($p \leq 0.05$) than static flexural modulus for paired specimens.
3. Irrespective of material, cyclic flexural strength was significantly higher ($p \leq 0.05$) than static flexural strength for paired specimens.
4. There was no effect of loading (cyclic versus static) on the qualitative fracture morphology across materials.

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REFERENCES

1. Haj-Ali R, Walker MP, Williams K. Survey of general dentists regarding posterior restorations, selection criteria, and associated clinical problems. *Gen Dent*. 2005; 53(5):369–75. *passim*. [PubMed: 16252541]
2. Manhart J, Chen H, Hamm G, Hickel R. Buonocore Memorial Lecture. Review of the clinical survival of direct and indirect restorations in posterior teeth of the permanent dentition. *Oper Dent*. 2004; 29(5):481–508. [PubMed: 15470871]
3. Brunthaler A, König F, Lucas T, Sperr W, Schedle A. Longevity of direct resin composite restorations in posterior teeth. *Clin Oral Invest*. 2003; 7(2):63–70.
4. Söderling, E. Practical Aspects of Salivary Analyses. In: Tenovou, JO., editor. *Human Saliva: Clinical Chemistry and Microbiology*. CRC Press, Inc.; Boca Raton: 1989. p. 6-19.
5. Adams SH 2nd, Zander HA. Functional tooth contacts in lateral and in centric occlusion. *J Am Dent Assoc*. 1964; 69(4):465–73. [PubMed: 14198784]
6. Neill DJ, Kydd WL, Nairn RI, Wilson J. Functional loading of the dentition during mastication. *J Prosthet Dent*. 1989; 62(2):218–28. [PubMed: 2760864]
7. Yurkstas AA. The masticatory act. a review. *J Prosthet Dent*. 1965; 15(2):248–62. [PubMed: 14267314]
8. Tortopidis D, Lyons MF, Baxendale RH, Gilmour WH. The variability of bite force measurement between sessions, in different positions within the dental arch. *J Oral Rehabil*. 1998; 25(9):681–6. [PubMed: 9758398]
9. Johnsen SE, Svensson KG, Trulsson M. Forces applied by anterior and posterior teeth and roles of periodontal afferents during hold-and-split tasks in human subjects. *Exp Brain Res*. 2007; 178(1): 126–134. [PubMed: 17031682]
10. Kim KH, Okuno O. Microfracture behaviour of composite resins containing irregular-shaped fillers. *J Oral Rehabil*. 2002; 29(12):1153–9. [PubMed: 12472851]
11. Kovarik RE, Ergle JW. Fracture toughness of posterior composite resins fabricated by incremental layering. *J Prosthet Dent*. 1993; 69(6):557–60. [PubMed: 8320639]
12. Iwamoto N, Ruse ND. Fracture toughness of human dentin. *J Biomed Mater Res A*. 2003; 66(3): 507–12. [PubMed: 12918033]
13. Kruzic JJ, Nalla RK, Kinney JH, Ritchie RO. Crack blunting, crack bridging and resistance-curve fracture mechanics in dentin: effect of hydration. *Biomaterials*. 2003; 24(28):5209–21. [PubMed: 14568438]

14. Nalla RK, Imbeni V, Kinney JH, Staninec M, Marshall SJ, Ritchie RO. In vitro fatigue behavior of human dentin with implications for life prediction. *J Biomed Mater Res A*. 2003; 66(1):10–20. [PubMed: 12833426]
15. Arola DD, Reprogl RK. Tubule orientation and the fatigue strength of human dentin. *Biomaterials*. 2006; 27(9):2131–40. [PubMed: 16253323]
16. Arola D, Zheng W, Sundaram N, Rouland JA. Stress ratio contributes to fatigue crack growth in dentin. *J Biomed Mater Res A*. 2005; 73(2):201–12. [PubMed: 15744763]
17. Hargreaves AS. The effects of cyclic stress on dental polymethylmethacrylate. II. Flexural fatigue. *J Oral Rehabil*. 1983; 10(2):137–51. [PubMed: 6573463]
18. Drummond JL. Cyclic fatigue of composite restorative materials. *J Oral Rehabil*. 1989; 16(5):509–20. [PubMed: 2809853]
19. McCabe JF, Wang Y, Braem M. Surface contact fatigue and flexural fatigue of dental restorative materials. *J Biomed Mater Res*. 2000; 50(3):375–80. [PubMed: 10737879]
20. Lohbauer U, von der Horst T, Frankenberger R, Kramer N, Petschelt A. Flexural fatigue behavior of resin composite dental restoratives. *Dent Mater*. 2003; 19(5):435–40. [PubMed: 12742440]
21. Brunski JB. Biomechanics of oral implants: future research directions. *J Dent Educ*. 1988; 52(12):775–87. [PubMed: 3057023]
22. Arola D, Reprogl RK. Effects of aging on the mechanical behavior of human dentin. *Biomaterials*. 2005; 26(18):4051–61. [PubMed: 15626451]
23. De Munck J, Van Landuyt KL, Coutinho E, Poitevin A, Peumans M, Lambrechts P, Braem M, Van Meerbeek B. Fatigue resistance of dentin/composite interfaces with an additional intermediate elastic layer. *Eur J Oral Sci*. 2005; 113(1):77–82. [PubMed: 15693833]
24. De Munck J, Braem M, Wevers M, Yoshida Y, Inoue S, Suzuki K, Lambrechts P, Van Meerbeek B. Micro-rotary fatigue of tooth-biomaterial interfaces. *Biomaterials*. 2005; 26(10):1145–53. [PubMed: 15451634]
25. ISO. ISO 4049:2000(E): Dentistry--Polymer-based Filling, Restorative and Luting Materials. 2000.
26. Asmussen E, Peutzfeldt A. Flexural strength and modulus of a step-cured resin composite. *Acta Odontol Scand*. 2004; 62(2):87–90. [PubMed: 15198388]
27. Goodis HE, Marshall GW Jr, White JM, Gee L, Hornberger B, Marshall SJ. Storage effects on dentin permeability and shear bond strengths. *Dent Mater*. 1993; 9(2):79–84. [PubMed: 8595846]
28. Strawn SE, White JM, Marshall GW, Gee L, Goodis HE, Marshall SJ. Spectroscopic changes in human dentine exposed to various storage solutions--short term. *J Dent*. 1996; 24(6):417–23. [PubMed: 8990687]
29. Habelitz S, Marshall GW Jr, Balooch M, Marshall SJ. Nanoindentation and storage of teeth. *J Biomech*. 2002; 35(7):995–8. [PubMed: 12052404]
30. Yap AU, Chandra SP, Chung SM, Lim CT. Changes in flexural properties of composite restoratives after aging in water. *Oper Dent*. 2002; 27(5):468–74. [PubMed: 12216565]
31. Bayne, SC.; Thompson, JY. *Biomaterials*. In: Roberson, TM.; Heymann, HO.; Swift, EJ., editors. *Sturdevant's art and science of operative dentistry*. Mosby Inc; St. Louis, MO: 2006. p. 196-205.
32. Anderson DJ. Measurement of stress in mastication. I. *J Dent Res*. 1956; 35(5):664–70. [PubMed: 13367282]
33. Gibbs CH, Mahan PE, Lundeen HC, Brehnan K, Walsh EK, Sinkewicz SL, Ginsberg SB. Occlusal forces during chewing--influences of biting strength and food consistency. *J Prosthet Dent*. 1981; 46(5):561–7. [PubMed: 6946223]
34. Shinogaya T, Bakke M, Thomsen CE, Vilmann A, Matsumoto M. Bite force and occlusal load in healthy young subjects--a methodological study. *Eur J Prosthodont Restor Dent*. 2000; 8(1):11–5. [PubMed: 11307384]
35. De Boever JA, McCall WD Jr, Holden S, Ash MM Jr. Functional occlusal forces: an investigation by telemetry. *J Prosthet Dent*. 1978; 40(3):326–33. [PubMed: 279679]
36. Yurkstas A, Manly RS. Measurement of occlusal contact area effective in mastication. *Am J Orthod*. 1949; 35(3):185–95. [PubMed: 18124177]
37. Woda A, Mishellany A, Peyron MA. The regulation of masticatory function and food bolus formation. *J Oral Rehabil*. 2006; 33(11):840–9. [PubMed: 17002744]

38. Walker MP, Spencer P, Eick JD. Mechanical property characterization of resin cement after aqueous aging with and without cyclic loading. *Dent Mater*. 2003; 19(7):645–52. [PubMed: 12901990]
39. Sideridou ID, Achilias DS. Elution study of unreacted Bis-GMA, TEGDMA, UDMA, and Bis-EMA from light-cured dental resins and resin composites using HPLC. *J Biomed Mater Res B Appl Biomater*. 2005; 74(1):617–26. [PubMed: 15889433]
40. Graf H. Bruxism. *Dent Clin North Am*. 1969; 13(3):659–65. [PubMed: 5256151]
41. Ahlgren J. Mechanism of mastication. *Acta Odontol Scand*. 1966; 24((suppl)44):22–27.
42. Anderson DJ, Picton DC. Tooth contact during chewing. *J Dent Res*. 1957; 36(1):21–6. [PubMed: 13398499]
43. Nanci, A. Ten cate's oral histology: development, structure, and function. Mosby, Inc.; St. Louis: 2003. Dentin-Pulp complex; p. 192-239.
44. Balooch M, Wu-Magidi IC, Balazs A, Lundkvist AS, Marshall SJ, Marshall GW, Siekhaus WJ, Kinney JH. Viscoelastic properties of demineralized human dentin measured in water with atomic force microscope (AFM)-based indentation. *J Biomed Mater Res*. 1998; 40(4):539–44. [PubMed: 9599029]
45. Carden A, Rajachar RM, Morris MD, Kohn DH. Ultrastructural changes accompanying the mechanical deformation of bone tissue: a Raman imaging study. *Calcif Tissue Int*. 2003; 72(2): 166–75. [PubMed: 12469250]
46. Elyashevich GK, Karpov EA, Lavrentiev VK, Poddubny VI, Genina MA, Zabashta YF. Changes in the amorphous phase of polyethylene upon high extension. *Int J Polymeric Mater*. 1993; 22(1-4):191–199.
47. Seguela R. Critical review of the molecular topology of semicrystalline polymers: The origin and assessment of intercrystalline tie molecules and chain entanglements. *J Polymer Sci Part B: Polymer Physics*. 2004; 43:1729–1748.
48. Negahban M, Wineman AS, Ma R-J. Simulation of mechanical response in polymer crystallization. *Int J Eng Sci*. 1993; 31(1):93–113.
49. Scheirs, J. Compositional and failure analysis of polymers: a practical approach. John Wiley & Sons, Ltd.; Chichester: 2000. Mechanical failure mechanisms of polymers; p. 304-362.
50. Toki S, Sics I, Ran S, Liu L, Hsiao BS, Murakami S, Tosaka M, Kohjiya S, Poompradub S, Ikeda Y. Strain-induced molecular orientation and crystallization in natural and synthetic rubbers under uniaxial deformation by in-situ synchrotron x-ray study. *Rubber Chem Technol*. 2004; 77(2):317–335. others.
51. Spencer P, Wang Y, Walker MP, Wieliczka DM, Swafford JR. Interfacial chemistry of the dentin/adhesive bond. *J Dent Res*. 2000; 79(7):1458–63. [PubMed: 11005728]

Figure 1.

Diagram of sectioning of composite beams in relation to polymerization intervals. The rectangles on the left side of the figure represent the small beams, as sectioned from a larger disc. The numbers 1-9 represent one surface's irradiation sites (9 irradiation sites exist on the other side of the disc as well). The letters A-D represent the four irradiations performed on the new surfaces after sectioning.

Figure 2.
Schematic representation of the three types of specimens and the origin of dentin within the dentin and laminate specimens.

Figure 3.
Schematic of the three-point bend fixture.

Figure 4.

Representative load-displacement diagrams for composite specimens, static (gray) and 60k-cyclic (black). To provide consistent modulus comparisons across static and cyclic specimens, the modulus was measured from 0-5N (arrow). This followed the cyclic multiple modulus measurement protocol in which the same specimen was stressed to ~25MPa (5N) after 1, 1k, 5k, 15k, and 35k loading cycles with modulus measured from the associated load-displacement diagrams.

Figure 5.

SEM micrographs of dentin beams exposed to cyclic and static loading conditions. Cyclic loading is represented by letters a through c, static loading by d through f. (a) and (d): 50X magnification, 25° tilt. (b) and (e): 1000X. (c) and (f): 5000X.

Figure 6.

SEM micrographs of composite beams exposed to cyclic and static loading conditions.

Cyclic loading is represented by letters a through c, static loading by d through f. (a) and (d): 50X magnification, 25° tilt. (b) and (e): 1000X. (c) and (f): 5000X.

Figure 7.

SEM micrographs of laminate beams exposed to cyclic and static loading conditions. Cyclic loading is represented by letters a through f, static loading by g through l. (a) and (g): 50X magnification, 25° tilt. (b) and (h): dentin, 1000X. (c) and (i): dentin, 5000X. (d) and (j): adhesive, 1000X. (e) and (k): composite, 1000X. (f) and (l): composite, 5000X.

Table 1

Mean Incremental Cyclic Flexural Modulus (GPa) and Standard Deviations

Material	After 1 Cycle	After 1k Cycles	After 5k Cycles	After 15k Cycles	After 35k Cycles	After 60k Cycles	Means Across Cycles
Dentin	6.96* (2.19)	7.29 (2.30)	8.64 (3.13)	8.01 (3.14)	9.34 (2.80)	10.57* (3.38)	8.47
Composite	6.11* (0.57)	6.55 (0.65)	7.41 (1.59)	7.18 (1.99)	8.80 (3.33)	8.23* (1.83)	7.38
Laminate	6.32* (1.53)	6.56 (1.57)	7.56 (1.41)	7.89 (1.34)	7.52 (2.35)	9.49* (1.97)	7.49
Means Across Material	6.32*	6.80	7.87	7.69	8.55	9.43*	

* Significant effect of cyclic loading ($p \leq 0.05$) on incremental flexural modulus measurements across materials. Difference significant only between 1 and 60k loading cycles.

Table II

Mean Flexural Modulus (GPa) and Flexural Strength (MPa) and Standard Deviations for Cyclic and Static Loaded Specimens

Material	Cyclic Flexural Modulus *	Static Flexural Modulus *	Cyclic Flexural Strength *	Static Flexural Strength *
Dentin	10.57 (3.38)	5.79 (1.96)	200 (39)	179 (26)
Composite	8.23 (1.83)	5.40 (0.64)	184 (16)	163 (17)
Laminate	9.49 (1.97)	5.29 (2.20)	180 (31)	163 (38)

* Significant effect of main effect of loading ($p \leq 0.05$) on flexural modulus and flexural strength across materials