Effect of amylase activity on mechanical properties of dental composite resin

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The purpose of this study was to investigate the effect of amylase activity on the mechanical properties of three dental composite resins (Filtek Z350; Filtek Z250 and Polofil Supra). The specimens were immersed in amylase solution at four different activities (25, 50, 100 and 200 KIU/L) with an artificial saliva solution (AS) as a control. The flexural strength (FS) and elastic modulus (EM) were determined in a three-point bending test after immersion for periods up to 12 months. In addition, surface roughness and surface morphology also determined. The FS of Filtek Z350 reaching the lowest level of 67.86 MPa after AS immersion for one year. There was a general trend for FS to decrease following immersion in AS for all the tested materials. However, the amylase groups did not undergo a significant decrease in FS and EM, and there was a slight increase in FS and EM for Polofil Supra.

**Keywords**: Composites, Mechanical properties, Degradation, Amylase activity, Surface

INTRODUCTION

The use of dental composite as a restoration for posterior teeth is increasing worldwide. Fracture is the primary reason for the replacement of filled resin restorations. In a review published in 2005, Sarrett et al. asserted that secondary caries and restoration fractures were the most common clinical problems in posterior composite restorations. Brunthaler et al. surveyed prospective studies on the clinical performance of posterior resin composites published between 1996 and 2002, and concluded that restoration fracture was the most common reason for failure within 5 years, followed by secondary caries. The fracture was also the predominant reason for failure in larger composite restorations older than 11 years. While recurrent decay was previously the most common cause for restoration failure in load-bearing areas, the increased application of composites to larger multi-surface class II cavities has resulted in a higher incidence of restoration failure due to fracture. Also, Bernardo et al. reported that main reason for failure was secondary caries, followed by fractures. While some data does not support this conclusion, it is clear that restoration fracture and secondary caries are the most common reasons for clinical composite resin failure.

The long-term clinical performance of dental composites is affected by several interacting variables such as water sorption, chemical degradation, biodegradation and fatigue. The salivary enzymes seem to play a key role in the biodegradation of dental materials. For example, salivary esterase shows strong degradative activity towards the methacrylate-based organic matrix of dental composite resin, and can catalyze the breakdown of bisphenol A-glycidyl methacrylate (Bis-GMA), one of the most common monomers used in dental composites and adhesives, to form the degradation product 2,2-bis [4 (2,3-hydroxy-propoxy) phenyl], resulting in a significant reduction in the bond strength and mechanical properties of composite resin. Amylase is the most abundant enzyme in human saliva, and the secretion of amylase is considered to be a biomarker of stress. Variation in starch intake correlates with the number of copies of the salivary amylase gene and amylase activity in saliva in human populations. Amylase is an essential component of salivary pellicle, and has been shown to be immobilized in an active conformation on intraorally exposed surfaces within 1 to 3 min. The role of salivary amylase in the pellicle has been discussed in the literature, mainly in relation to microorganisms involved in starch digestion that can form complexes with high molecular weight mucins (MG1). However, limited information is available about the influence, especially the long-term effects, of salivary amylase activity on the mechanical properties of dental composite resin.

We are working to clarify the effects of changes in the activity of the salivary enzymes especially salivary amylase activity by stress on the longevity of the restorative materials. This study as part of these studies, and is a pilot study to investigate the effect of amylase activity on the durability of dental composite resins.

The purpose of this study was to investigate the
effects of amylase activity on the flexural strength (FS), elastic modulus (EM) and surface properties of three dental composite resins. We hypothesized that amylase activity will affect the mechanical properties of composite resin more than artificial saliva, and that high levels of amylase activity will reduce the FS and EM; and roughened the surface of composite resin.

MATERIALS AND METHODS

One nanofilled composite resin (Filtek Z350), and two microhybrid composite resins (Filtek Z250 and Polofil Supra) were selected. Details of the materials are shown in Table 1. A stainless-steel mold was used to prepare test specimens 25×2.0×2.0 mm. The mold was slightly overfilled with composite resin and the material was pressed down with a glass microscope slide. The material was light cured according to the manufacturer's instruction (Curing time: 20 s for Filtek Z250 and Filtek Z350, and 60 s for Polofil Supra) with a polymerization unit (Pencure, Light intensity: 1,000 mW/cm²; J Morita MFC, Tokyo, Japan). After curing, the specimens were removed from the mold and any flash material was trimmed away with 600 grit sandpaper, leaving the surfaces untouched. The thickness of the specimens was measured by caliper. Only specimens with a thickness of 2.0±0.05 mm were accepted. Each specimen was then inspected on a light box to ensure that no porosity was present, and stored in distilled water at 37°C for 24 h.

The flexural strength (FS) and elastic modulus (EM) at the maximum load were determined in a three-point bending test (n=5) according to ISO 4049:2009. And the elastic modulus (EM) was calculated using the following equation: EM=3Flbh3d (F is the maximum load; l is the deflection at load F; b is the distance between the supports; b is the width of specimen; h is the height of the specimen). The specimens were then immersed in amylase solution (dissolve the amylase powder to distilled water; α-amylase, Lot: LAE4677, Cat. No.: 013-03732, Wako, Osaka, Japan) (AM) at four different concentrations: 0, 10, 25, and 50 KIU/L, with artificial saliva solution did not contain the amylase activity. The specimens were then immersed in 25 KIU/L saliva induced a significant decrease in flexural strength. There was no significant difference in the flexural strength of the materials when immersed in 25 KIU/L saliva.

The flexural strength and elastic modulus were determined using a universal testing machine (Model 5565; Instron, Canton, MA, USA). Apply a load to the specimen at a cross-head speed of 1.0 mm/min until the specimen reaches the yield point or fracture according to ISO 4049:2009 after immersion for 0 days, 1 month, 2 months, 3 months, 6 months and 12 months. Then record the maximum load at the yield point or fracture, and calculate the FS and EM using the above formulation.

After immersion for 12 months, the surface roughness (R̴) of five specimens of each material was measured by means of a contact stylus profilometer (SJ-400, Mitutoyo, Kawasaki, Japan) with a diamond stylus (tip radius: 2 μm, load: 0.75 mN; tracing length: 2.5 mm; stylus speed: 0.5 mm/s, cut-off length: 0.8 mm). Three measurements were performed for each worn specimen. The roughness parameter for each material was evaluated as the arithmetic mean over the 15 measurements.

One specimen of each material after immersion for 12 months was sputter coated with gold using a JFC-1600 sputtering device (JEOL, Tokyo, Japan) and examined by SEM (JSM-5510, JEOL) using secondary emission electron imaging at 1,000-fold magnification.

Results were statistically analyzed by one-way and three-way analysis of variance (ANOVA) and Tukey’s HSD test (α=0.05), allowing a comparison of the FS and EM among the different materials, amylase activities and immersion times. The mean volume surface roughness of each material was analyzed by one-way ANOVA and Tukey’s HSD test (α=0.05).

RESULTS

Table 1 Materials tested

| Material   | Manufacturer | Lot      | Matrix resin         | Filler                | Type            | Filler content (vol%) |
|------------|--------------|----------|----------------------|-----------------------|------------------|-----------------------|
| Filtek Z350| 3M, St. Paul, MN, USA | N313029 | Bis-GMA, UDMA, TEGDMA, Bis-EMA | zirconia/silica 5–20 nm, silica 20 nm. | nanofilled | 59.5 |
| Filtek Z250| 3M           | N147363 | Bis-GMA, UDMA, Bis-EMA | zirconia/silica 0.01–3.5 μm. | microhybrid | 60.0 |
| Polofil Supra| Voco, Cuxhaven, Germany | 1119146 | Bis-GMA, TEGDMA, Diurethanedimethacrylate | inorganic filler (0.05 μm, 0.5–2 μm) | microhybrid | 60.0 |

Three-way ANOVA shows that both the material type and immersion time have a significant impact on flexural strength and the elastic modulus, while amylase activity has a distinct influence on the elastic modulus but no effect on flexural strength (p=0.566).

The flexural strength of materials was shown in Table 2 and Figs. 1a–c. The influence of amylase is specific to the material used. For Filtek Z250, artificial saliva induced a significant decrease in flexural strength. There was no significant difference in the flexural strength of the materials when immersed in 25 KIU/L saliva.
or 50 KIU/L amylase for one year (Table 2 and Fig. 1a). Activities of 100 KIU/L and 200 KIU/L amylase induced a significant decrease in flexural strength after one or two months of immersion than before immersion, followed by an increase. However, the highest value of flexural strength was indicated than specimen that immersed for one year. The elastic modulus was significantly decreased when the materials were immersed in 25 KIU/L amylase for one year (Table 3 and Fig. 2a–c). For Filtek Z250, the specimens of flexural strength irrespective of the amylase activity induced a significant decrease in flexural strength, while immersion in amylase induced significant increases in flexural strength irrespective of the amylase activity (Table 2 and Fig. 1c).

The elastic modulus of materials was indicated in Table 3 and Fig. 2a–c. For Filtek Z250, the specimens of elastic modulus decreased significantly when the materials were immersed in artificial saliva had no significant difference over the entire test period (Table 3 and Fig. 2a). The elastic modulus decreased significantly when the materials were immersed in 25 KIU/L amylase for one year. The elastic modulus was significantly decreased at an activity of 200 KIU/L amylase, but there was no significant change at an activity of 100 KIU/L amylase (Table 3 and Fig. 2a). The elastic modulus of Filtek Z350 decreased significantly in the artificial saliva specimens.

### Table 2 Flexural strength (MPa) of materials with immersion time

| Materials | Solutions | 0 M | 1 M | 2 M | 3 M | 6 M | 12 M |
|-----------|-----------|-----|-----|-----|-----|-----|------|
| Filtek Z250 | AS 25 KIU/L | 167.55±18.20<sup>a</sup> | 143.61±24.85<sup>b</sup> | 114.76±21.07<sup>c</sup> | 124.32±18.66<sup>d</sup> | 127.99±9.59<sup>e</sup> | 106.76±7.67<sup>f</sup> |
| Filtek Z350 | 25 KIU/L | 168.03±37.46<sup>a</sup> | 146.81±19.39<sup>a</sup> | 159.84±30.84<sup>a</sup> | 148.13±14.08<sup>a</sup> | 171.48±9.04<sup>bc</sup> | 156.77±18.00<sup>bc</sup> |
| Polofil Supra | 25 KIU/L | 170.92±30.79<sup>a</sup> | 130.99±23.54<sup>a</sup> | 140.30±28.40<sup>a</sup> | 126.35±21.33<sup>a</sup> | 156.92±24.48<sup>a</sup> | 133.62±21.14<sup>bc</sup> |
| Filtek Z250 | 50 KIU/L | 169.93±13.62<sup>a</sup> | 132.53±17.95<sup>a</sup> | 146.34±14.71<sup>a</sup> | 148.37±16.90<sup>a</sup> | 187.17±13.91<sup>e</sup> | 175.72±19.76<sup>de</sup> |
| Filtek Z350 | 50 KIU/L | 179.91±18.13<sup>a</sup> | 166.50±23.02<sup>a</sup> | 138.30±32.05<sup>a</sup> | 156.88±16.49<sup>a</sup> | 153.68±5.09<sup>b</sup> | 168.92±9.52<sup>d</sup> |
| Polofil Supra | 50 KIU/L | 164.00±17.66<sup>a</sup> | 136.38±28.83<sup>a</sup> | 142.01±9.65<sup>a</sup> | 151.47±35.46<sup>a</sup> | 143.43±16.70<sup>a</sup> | 156.71±10.73<sup>b</sup> |
| Filtek Z250 | 100 KIU/L | 140.00±17.66<sup>a</sup> | 136.38±28.83<sup>a</sup> | 142.01±9.65<sup>a</sup> | 151.47±35.46<sup>a</sup> | 143.43±16.70<sup>a</sup> | 156.71±10.73<sup>b</sup> |
| Filtek Z350 | 100 KIU/L | 149.19±18.18<sup>a</sup> | 130.58±26.17<sup>a</sup> | 142.01±9.65<sup>a</sup> | 151.47±35.46<sup>a</sup> | 143.43±16.70<sup>a</sup> | 156.71±10.73<sup>b</sup> |
| Polofil Supra | 100 KIU/L | 126.88±22.32<sup>a</sup> | 131.65±40.42<sup>a</sup> | 148.66±18.34<sup>a</sup> | 154.25±35.46<sup>a</sup> | 148.66±18.34<sup>a</sup> | 151.47±35.46<sup>a</sup> |
| Filtek Z250 | 200 KIU/L | 140.00±17.66<sup>a</sup> | 136.38±28.83<sup>a</sup> | 142.01±9.65<sup>a</sup> | 151.47±35.46<sup>a</sup> | 143.43±16.70<sup>a</sup> | 156.71±10.73<sup>b</sup> |
| Filtek Z350 | 200 KIU/L | 149.19±18.18<sup>a</sup> | 130.58±26.17<sup>a</sup> | 142.01±9.65<sup>a</sup> | 151.47±35.46<sup>a</sup> | 143.43±16.70<sup>a</sup> | 156.71±10.73<sup>b</sup> |
| Polofil Supra | 200 KIU/L | 126.88±22.32<sup>a</sup> | 131.65±40.42<sup>a</sup> | 148.66±18.34<sup>a</sup> | 154.25±35.46<sup>a</sup> | 148.66±18.34<sup>a</sup> | 151.47±35.46<sup>a</sup> |

<sup>a</sup>Identical letters indicate no significant differences (Tukey's HSD test, p>0.05)
<sup>b</sup>abc: between the solutions (same materials and same immersion times); αβγ: between the time (same materials and same solutions)

![Fig. 1](image-url) Variation in flexural strength of materials with immersion time (n=5).

a: Filtek Z250; b: Filtek Z350; c: Polofil Supra
Table 3  Elastic modulus (GPa) of materials with immersion time

| Materials | Solutions | AS  | 25 KIU/L | 50 KIU/L | 100 KIU/L | 200 KIU/L |
|-----------|-----------|-----|----------|----------|-----------|-----------|
| Filtek Z250 | 0 M | 15.39±2.89  
| Filtek Z350 | 0 M | 11.06±1.41  
| Polofil Supra | 0 M | 10.09±0.87  
| Filtek Z250 | 1 M | 13.24±1.13  
| Filtek Z350 | 1 M | 13.09±0.44  
| Polofil Supra | 1 M | 10.46±1.29  
| Filtek Z250 | 2 M | 12.84±0.65  
| Filtek Z350 | 2 M | 11.51±0.39  
| Polofil Supra | 2 M | 10.74±1.60  
| Filtek Z250 | 3 M | 13.97±0.51  
| Filtek Z350 | 3 M | 11.91±0.63  
| Polofil Supra | 3 M | 8.77±1.41   
| Filtek Z250 | 6 M | 14.36±0.55  
| Filtek Z350 | 6 M | 12.34±1.15  
| Polofil Supra | 6 M | 12.28±1.16  
| Filtek Z250 | 12 M | 14.26±0.91  
| Filtek Z350 | 12 M | 9.97±0.99   
| Polofil Supra | 12 M | 10.49±1.64  

*Identical letters indicate no significant differences (Tukey’s HSD test, \( p > 0.05 \))

*abc: between the solutions (same materials and same immersion times);  
aβγ: between the time (same materials and same solutions)
but increased slightly in the amylase specimens (Table 3 and Fig. 2b). The specimens of Polofil Supra, immersion in amylase induced significant increases in elastic modulus irrespective of the amylase activity (Table 3 and Fig. 2c). There was a slight decrease in the elastic modulus after immersion for 3 months in artificial saliva, followed by an increase.

The mean values and standard deviations of surface roughness of the tested materials are summarized in Fig. 3. In comparison with before immersion, the surface roughness of all materials showed almost same values except the sample of Polofil Supra immersed in 50 KUI/L amylase. The surface roughness of Polofil Supra immersed in 50 KUI/L amylase showed highest
value than samples immersed in other amylase activity solutions (Fig. 3). Roughness was apparently material-dependent and amylase activity-dependent.

SEM representative photographs of the tested materials after immersion for 12 months are presented in Figs. 4–6. All specimens immersed in different amylase activity solutions reveal similar surface scratching after immersion for 12 months.

**DISCUSSION**

Human saliva is a complex mixture of liquid and particulate matter. Major components of saliva are proteins, which include immunoglobulins and enzymes. One class of enzymes found in saliva is amylase, which to be involved in the degradation of starch. Salivary amylase identified as one of the major structural components of the acquired salivary pellicle on enamel and dental materials. Hannig et al. reported that amylase occurs in similar relative amounts and with similar relative enzyme activity on the different tested substrate, but with great intraindividual and interindividual differences. The present study investigated the effect of amylase activities and immersion time on the flexural strength and elastic modulus; and surface properties of composite resin. From our results, although the mechanical properties of commercial composite resin were affected by amylase activity, however, this effect was less than that of artificial saliva. And the surface roughness of materials was not affected by the amylase activity. Therefore, our hypothesis was rejected.

The longevity of dental composite resin is a major problem in operative dentistry. In the clinic, the mechanical properties of composite resin may be the most important factor determining a doctor's acceptance. This study examined changes in the flexural strength, elastic modulus and surface roughness of three dental composite resins caused by amylase activity. In this study, four different amylase activities (25 KIU/L, 50 KIU/L, 100 KIU/L and 200 KIU/L) were used as the immersion solution. Several investigators have reported that salivary amylase activity could be a useful tool for evaluating stress. When the human being feels stress, salivary amylase activity will be rises. This range is between 20–250 KUL/L.

Physicochemical surface characteristics such as surface free energy, electrical charge, composition, microstructure, and surface roughness may affect the adsorption and enzyme activity of amylase on solid surfaces, and may induce varying results for different materials.

It is well established that exposure of composite resin to an aqueous environment diminishes its mechanical properties, predominantly due to uptake of water by the polymer. Absorbed water causes sorption expansion, increasing the effective free volume and the ease of movement of chain segments, thus reducing the elastic modulus of the material. Hydrolytic degradation of the polymer matrix and failure of the filler-matrix bond in an aqueous environment also reduces flexural strength and elastic modulus. Previous studies have shown that the properties of composite resin may be significantly diminished within 2 or 3 months aging in water.

And also Shah et al. reported that the flexural strength degradation may due to degradation of resin, the filler or the resin/filler interface. Although the grain size, shape, size distribution, and the adhesion between the grains can affect the strength of composite resin. And larger grain size generally gives higher peak toughness than small gran size. In this study, flexural strength degradation of the nanofill composite resin (Filtek Z350) was significantly greater than microhybrit composite resin (Filtek Z250) (Table 1). In the present case, the reported filler particle size for the microhybrit composite resin is higher than for the nanofill composite resin (Table 1). This may have the reason for the different flexural strength degradation between the Filtek Z250 and Filtek Z350. And also interfacial debonding may occur in the nanofill composite resin.

ISO 4049 requires the type I filling resin —filling for the occlusal areas, flexural strength ≥80 MPa. Both microhybrit materials (Filtek Z250 and Polofil Supra) met all requirements before and after aging; however, the flexural strength of the nanofilled material (Filtek Z350) was only 67.86 MPa after immersion for one year in artificial saliva —lower than the ISO requirement. Filtek Z350 has a similar resin matrix and filler loading to Filtek Z250 and Polofil Supra, but its filler particle size is quite different, and this may account for the difference in its ability to resist aging in artificial saliva.

The elastic modulus refers to the relative stiffness of a material. The elastic modulus and other mechanical properties of composite resin are important in determining their resistance to occlusal forces. A material with a low modulus of elastic placed in a load-bearing area will result in a higher deformability under masticatory stress, and possible catastrophic failure. For occlusal stress bearing restorations, the modulus of elastic must be high to withstand occlusal forces and deformation.

According to our findings, the type of material, immersion time and amylase activity all have a significant impact on the elastic modulus (Table 3). According to Curtis et al., a larger surface area to volume ratio of the nanofiller in the nanofilled composite tends to increase water uptake, resulting in degradation of the filler/matrix interface and affecting the mechanical properties. The long-term viability of new nanofilled composite resin has not yet been confirmed.

Filtek Z350 is a nanofilled composite. The main component of organic matrix is Bis-GMA and the triethyleneglycol dimethacrylate (TEGDMA). Filler content is 59.5 v/v% according to the manufacturer. The Filtek Z250 and Polofil Supra are the microhybrit composites with 60 v/v% filler content according to manufacture. Polofil Supra main component of organic matrix is Bis-GMA and the TEGDMA same as Filtek Z350. However, main component of organic matrix of Filtek Z250 is only Bis-GMA. We thought that this
difference in main component is a reason that indicated the different effects of amylase activities on the mechanical properties of dental composite resins.

In the present study, there was a general trend for flexural strength to decrease following immersion in artificial saliva for all the tested materials. However, the amylase groups did not undergo a significant decrease in flexural strength and elastic modulus, and there was a slight increase in flexural strength and elastic modulus for Polofil Supra, which may be attributed to the protective properties of amylase as it adsorbs to the surface of the material and prevents degradation by water. Another possible explanation is that artificial saliva has a greater aging effect on composite resin than water. Söderholm et al. observed that composite resin leached more filler material on exposure to artificial saliva than water. Artificial saliva has a significant impact on the elastic modulus, which decreases with aging time. However, amylase activity has limited influence on the elastic modulus.

The present study did not completely simulate clinical behavior because all specimens were tested in a dry state. To overcome the limitations of the in vitro tests, the flexural strength of composite resin actually used by patients should be evaluated; and the salivary amylase activity of patients should be measured. Thermocycling and cyclic loading are recommended in further studies to better simulate intraoral conditions. Furthermore, in order to understand the influence of amylase activity on mechanical properties it is necessary to study how amylase activity influences other properties of these materials, such as flexural toughness, hardness, surface properties, dimensional change, leachability of components, water sorption and solubility.

CONCLUSIONS

The present study suggests that amylase activity can effectively prevent deterioration in the mechanical properties (flexural strength and elastic modulus) of dental composite resin in an aqueous environment. And the surface roughness of materials was not affected by the amylase activity. However, the effect of the amylase activity varied according to the composite resin. Differences in material type, filler type and content could be responsible for this variation in influence. The flexural strength of nanofilled composite after aging and flexural toughness of composite resin needs to be further investigated.

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