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Fracture resistance and flexural strength of endodontically treated teeth restored by different short fiber resin composites: a preclinical study

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Abstract

Aim: To evaluate the effect of using different short fiber-reinforced resin composites on fracture resistance and flexural strength of endodontically treated tooth and compare it with nano-filled resin composite.

Methods: Sixty human premolars were used for the fracture resistance test. Root canals were sequentially enlarged using a Pro-taper system from SX to F3 and obturated with Gutta-percha. Mesio-Occluso-Distal (MOD) cavities were prepared in all teeth. Teeth were then divided into 3 groups (n = 20 each) based on the type of resin composite. Group 1: Alert fiber-reinforced resin composite, Group 2: EverX Flow fiber-reinforced resin composite, and Group 3: Z350 nano-filled resin composite. Each group were subjected to a load till fracture using a universal testing machine to measure the fracture resistance. For the flexural strength test, 10 specimens from each material were prepared and 3- point bending tests were performed. The results of both tests were analyzed by using Weibull analysis.

Results: Teeth restored with Ever X fiber-reinforced resin composite conveyed the highest significant fracture resistance and flexural strength value when compared to the other two materials.

Conclusions: Short Fiber-reinforced resin composite can be considered a better choice for the restoration of MOD cavities in endodontically treated teeth.

Keywords: Fracture resistance, Endodontic treated teeth, Fiber-reinforced resin composite

Background

Endodontically treated teeth (ETT) are more prone to fracture when compared to vital teeth, the weakened structure of endodontically treated teeth was primarily referred to the loss of anatomical structures such as marginal ridges, cusps, and pulp chamber roof due to caries, access cavity, and radicular preparation that increase tooth fragility (Kalburge et al. 2013; Hannig et al. 2005).

Cavity preparation in non-vital teeth especially the preparation of the Mesio-Occluso-Distal cavities brings a further remarkable decrease in tooth strength owing to the extensive cavity preparation and the micro-fractures that result from the applied occlusal forces. Restoration of endodontically treated teeth especially those with extensive tooth loss is a true challenge for the operators. There have been several materials developed for the restoration of endodontically treated teeth including amalgam, glass ionomer, and resin composite (Ozsevik et al.2015).

Resin-bonded composites can improve rigidity and increase the fracture resistance of non-vital teeth. Unluckily, polymerization shrinkage is still the major

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drawback for the use of this type of restoration, especially in large cavities, which consequently leads to tooth fracture and restoration failure (Taha et al. 2009).

To withstand this drawback in conventional resin composite, the addition of fibers of different sizes and morphology was done to modify the physical and mechanical properties of the resin composite leading to the development of a new composite under the name of Fiber-reinforced resin composites. The latter offers a reduction in polymerization shrinkage and consequantly, increases the toughness and impact strength. The fiber-reinforced resin composite was classified according to the type of fibers incorporated in the resin into a glass, carbon, or polyethylene fiber. Glass fiber-reinforced resin composite was frequently used for restoring endodontically treated teeth (Kumar et al. 2016; Goguță et al.2012).

Recently, the short fiber-reinforced resin composite was introduced as a dentine replacement. Its structure nearly resembles the fibrous structure of natural dentine. This material is mainly used as a bulk base in large restorations, especially in stress-bearing areas. The composition of these composites was mainly E- glass short fibers and inorganic fillers embedded in an organic matrix. This structure enhances bonding properties and improves the toughness of the resin composite (Garoushi et al. 2018). Alert resin composite was the initial formulation of the short glass fiber-reinforced resin composite that was introduced into the markets in the late 1990s while a more recent formulation (Ever X Flow) was launched in the markets in 2019 (Lassila et al. 2020).

Fracture resistance is considered one of the main characteristics of dental materials as it describes the tolerance of the material to different stresses. Its values depend on the ability of the material to resist the crack propagation that originated from its internal defects which

subsequently leads to microscopic fractures in the margins of the restoration or even results in bulk fractures of the filling itself (Bonilla et al. 2001). Flexural strength (transverse strength) is another test with a combination of both compressive and tensile strength, it includes elements of proportional limit and modulus of elasticity measurements. Both fracture resistance and flexural strength are part of the criteria that can estimate the clinical longevity of the restoration (Paidi et al. 2017).

Since the process of development of new materials was very fast, the clinicians were often confused regarding the choice of the best material that can mimic the effect of natural structure. Hence, the present study aimed to evaluate the fracture resistance and flexural strength of endodontically treated teeth restored with two short fiber-reinforced resin composite restorations compared to nano-filled resin composite restoration. The Null hypothesis test is that no difference shows in fracture resistance and flexural strength between the 2 types of short fiber-reinforced composite and nano-filled resin composite when used to restore endodontically treated teeth.

Materials and methods

Materials used, compositions, and manufacturers are illustrated in Table 1.

Methods

Sample size calculation

The power of the study was evaluated using post-hoc analysis. Sixty samples (n=20 for each group) with a one-way ANOVA study achieves a power of 100%. The effect size f=1.3 and the significant level was set at 0.05 (G*Power, v3.1.9.7 for windows).

Table 1 Materials, compositions, and manufacturers

| Material | Specifications | Composition | Manufacturer | Batch number |
|-----------------------------|--|---|--|--------------|
| Alert (Alr) | Condensable fiber reinforced Composite | Bis-GMA, UDMA, TEGDMA, THFMA, Filler: Silica and micrometer-scale glass fiber 84% by weight, 62% by volume | Pentron, Wallingford, CT, USA https://www.pentron.com | 6931490 |
| EverX flow (Exf) | Short fiber reinforced flowable composite for dentin replacement | Bis-EMA, TEGDMA, UDMA, micrometer-scale glass fiber filler, Barium glass 70% by weight, 46% by volume | GC Co, Tokyo, Japan https://www.gc.dental.com | 2001241 |
| Filtek Z350 (FZ3) | Light-cured, nano-filled composite | 78.5% by weight (58–60% by volume) combination of aggregated zirconia/silica cluster filler | 3 M ESPE, Paul, USA https://www.3m.com | 7018A3B |
| G-ænial posterior composite | MFR hybrid composite | (UDMA) and dimethacrylate co- monomers, Fluoroaluminosilicate glass, fumed silica | Vita [®] , Säckingen, Germany | 1607271 |

Sample collection

Sixty intact single-rooted premolars that were freshly extracted for orthodontic purposes, using the luxation method, were collected from National Research Centre and used in this pre-clinical study. All teeth were examined under transillumination and magnification (2 × magnification lens) and only teeth that were free of cracks, caries, fractures, and without any previous restorations were included in the study. A hand scaler was used to remove any soft tissue and calculus deposits from the teeth, then all teeth were rinsed in distilled water and stored in a saline solution.

Sample preparation

Standardized endodontic access cavities were prepared in the teeth using 2# diamond round bur with coolant in a high-speed handpiece (Dentsply, Tulsa, USA) for penetration of the pulp chamber, and then tapering cylinder bur was used to complete the access cavity. For all specimens, MOD cavities were then prepared using cylindrical diamond burs size 2# (Diatech, Heerbrugg, Germany) by one operator with the following dimensions: The width of the occlusal isthmus was one-half of the inter-cuspal distance, the pulpal floor was 2.5 mm in depth, the axial wall depth was 1.5 mm, the width of the proximal box was half the buccolingual dimensions, the gingival floor was placed 1 mm above the cementoenamel junction. The bur was changed after each five prepared cavities.

A 10 k-file (Mani Inc, Japan) was used to establish the working lengths. The root canals were sequentially enlarged using a Pro-taper system from SX to F3 till the determined working length according to manufacturer guidelines using X Smart Endomotor (Dentsply-Maillefer, Ballaigues, Switzerland) Irrigation was performed using 3 mL of 2.5% NaOCl after every change of instrument. Following biomechanical preparation, 17% EDTA was used for 1 min, followed by distilled water for 1 min, and dried with medium paper points (Dentplus, Choonchong, Korea). After the complete preparation of the teeth, all the canals were obturated using Gutta-percha (Diadent, Group International, Korea) with the use of a cold lateral condensation technique and AD sealant (Meta Biomed, Cheongwon, Korea) (Moosavi et al. 2012). A hot instrument was used to remove excess gutta-percha from the coronal orifice of the canals, and all samples were stored for seven days in 100% humidity to allow for the setting of the sealer. After that, the pulp chamber was filled with resin-modified glass ionomer cement (GC Fuji II LC Capsule, GC Corporation) to a thickness that extended 1 mm occlusal to the cementoenamel junction (CEJ).

The Specimens were then divided randomly into three groups (n=20 each) according to the type of resin composite used for restoration, Group 1: Alert fiber-reinforced resin composite [Alr], Group 2: *Ever X flow* fiber-reinforced bulk fill flowable composite [Exf], and Group 3: Filtek Z350 nano-filled resin composite [FZ3].

After applying auto-matrices in all spacemen, the application of 37% phosphoric acid (Ivoclar Vivadent) was done for 30 s for enamel and 15 s for dentine followed by water rinsing for 20 s then air dryness was done for 5 s. A single bond (3 M ESPE, St. Paul, MN, USA) was applied followed by light curing with Optilux 500 (Demetron-Kerr, Orange, CA, USA) with a light intensity of 1200 mW/cm² for 10 s. In all cavities of Alert and Nanofilled composite, the first layer of resin composite (2 mm thick) was applied on the gingival seat of both proximal boxes in the MOD cavities and packed near the axial wall then light-cured for the 40 s. Subsequent layers of the same thickness were placed in a direction from the gingival floor to the occlusal surface to fill the preparation and each increment was then light-cured for the 40 s. Regarding Ever X flow resin composite application, it was applied into the cavity as one bulk layer to fill all the MOD cavities leaving only 1 mm from the occlusal surface, and then light-cured for the 40 s. A universal resin composite (GC Corporation Tokyo, Japan) was applied over the Ever X Flow for the thickness of 1 mm at the surface of the tooth and cured for 40 s. (Goda and Abogabal, 2020) (Fig. 1).

In all groups after the removal of matrix bands, postcuring was done from the mesial and distal surface for the 40 s according to manufacture instructions, then restorations were finished and polished using 12# finishing bur and rubber point at a low-speed handpiece. After that, all specimens were stored in distilled water for 24 h. The self-cure acrylic resin was used to fix all the spacemen in cylindrical tubes (5 mm \times 5 mm) in a direction parallel to the long axis of the tube, leaving 1 mm from the cementoenamel junction.

Fracture resistance testing

For the fracture resistance test, each group have mounted individually on a computer-controlled testing machine (Model 3345; Instron Industrial Products, Norwood, MA, USA) with a load cell of 5 k, N, then the readings were registered using computer software (Instron® Bluehill Lite Software). The spacemen were fixed by securing screws to the lower fixed chamber of the testing machine. A compressive load was applied occlusally by using a metallic cylindrical rod with a 3.8 mm round tip connected to the upper movable chamber of the testing machine traveling at a crosshead speed of 1 mm/min. The tip was parallel to the long axis of the teeth and touch 3 points on the

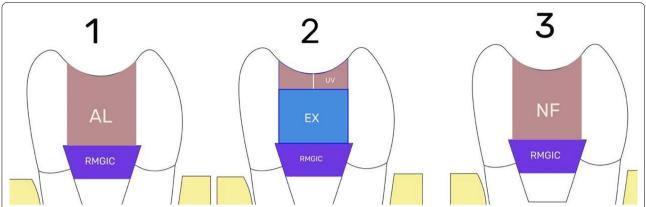


Fig. 1 Illustration of different restoration groups in tooth model: pulp chamber was filled with resin-modified glass ionomer cement (RMGIC) in the 3 groups. Group 1, coronal restoration with Alert fiber-reinforced resin composite (AL); Group 2, coronal restoration with Ever X flow fiber-reinforced (Ex) followed by 1 ml of universal composite (UC); and Group 3: coronal restoration with nano-filled resin composite (NF)

surface of the tooth: the occlusal surface, the buccal, and the lingual walls. The failure mode was manifested by a detectable crack and was established by a recorded sharp drop in the load–deflection curve detected on the computer software (Bluehill Lite Software Instron[®] Instruments). Fracture load was calculated in Newton.

Flexural strength testing

For testing flexural strength 10 specimens from each tested material were prepared according to the following methodology: Stainless steel mold with a dimension of $2.5 \text{ widths} \times 2 \text{ length} \times 2 \text{ height was used to fabricate the specimen according to ISO 4049 specifications. A glass slab was placed below the mold and then the restorative material was packed into the mold-covered over the celluloid strip to obtain a finished surface then another glass slab was placed over the mold with light pressure for the removal of excess material. Polymerization of each specimen was done using a light cure machine Optilux 500 ($ *Demetron-Kerr, Orange, CA, USA*) in the 40 s each. After curing, each specimen was stored in distilled water till tested within one week.

A three-point bending flexural strength test was done using a universal mechanical testing system (Instron Corp., Canton, MA). Each specimen was placed over a 2 parallel support (2 mm in diameter each and 20 mm apart). The load with a speed of 0.75 mm\min was then applied from a 2 mm rod placed in the center between the 2 supports. The maximum force (N) exerted on the specimen before being subjected to fracture was recorded. The following equation was used for the calculation of the flexural strength (σ):

$$\sigma = \frac{3ps}{2bh^2}$$

P: is the maximal force exerted on the specimens. S: is the distance between the supports (20 mm).

b and h are the widths in mm and the heights in mm of the specimen, respectively, measured immediately before testing.

Statistical analysis

Weibull analysis was used for statistically analyzing the Fracture Resistance (N) and Flexural Strength (MPa) data (R4, R Foundation for Statistical Computing, Vienna, Austria). Its parameters were calculated by Wald estimation, and pivotal confidence bounds were calculated with Monte Carlo simulation. The different groups were compared at the characteristic strength (63.2% probability of failure and 10% probability of failure) (α = 0.05).

Results

Results of fracture resistance and flexural strength are presented in Table 2 and Fig. 2. regarding fracture resistance, when using Weibull characteristic strength, no difference between all tested groups was found. However, when using P10 (estimation at 10% probability of failure) Exf showed a significantly higher fracture resistance compared to FZ3. For Flexural strength, Exf revealed a significantly higher Weibull characteristic strength compared to FZ3, while an insignificant difference between groups resulted in P10. Overall, for both fracture resistance and flexural strength, Exf showed the highest Weibull modulus which indicates reliable results for Exf.

Table 2 Weibull results of fracture strength and flexural strength

| | $Mean \pm SD$ | α (95% CI) | β (95% CI) | P10 (95% CI) |
|------------------|-------------------|----------------------------------|-------------------|--|
| Fracture resista | nce (N) | | | |
| Alr | 462.3 ± 119.5 | 506.1 (401.3–630.0) [§] | 4.4 (2.2–7.5) | 304.5 (202.4–458.2) ^{zΩ} |
| Exf | 594.1 ± 56.0 | 617.5 (571.1–664.6) [§] | 13.4 (6.4–23.3) | 521.9 (453.8–600.3) ^z |
| FZ3 | 435.2 ± 132.6 | 481.4 (363.6–627.9) [§] | 3.6 (1.8–6.0) | $258.6 (158.5-422.2)^{\Omega}$ |
| Flexural strengt | th (MPa) | | | |
| Alr | 74.6 ± 8.1 | 77.7 (70.5–85.7) ^{AB} | 12.3 (6.5–107.5) | 64.7 (41.3–74.7) ^A |
| Exf | 79.7 ± 5.0 | 81.6 (78.5–84.8) ^A | 26.4 (14.2-191.8) | 74.9 (62.7–79.5) ^A |
| FZ3 | 65.7 ± 5.4 | 68 (63–73.3) ^B | 13.8 (8–51.5) | 57.8 (43.2–64.8) ^A |

Different superscript letters or symbols inside the α column for each parameter are statistically significant differences based upon a 95% confidence interval (CI). α: characteristic strength or scale of a Weibull parameter. β: the shape, slope, and modulus of a Weibull parameter. P10: estimation at 10% probability of failure

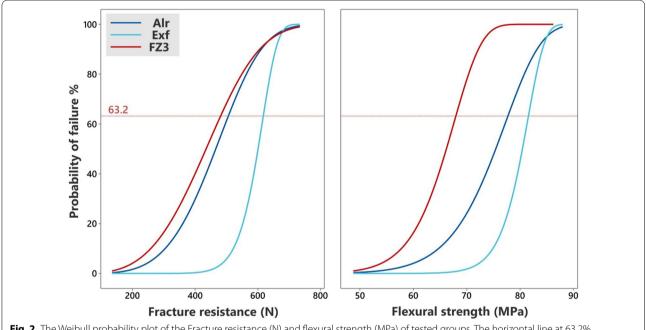


Fig. 2 The Weibull probability plot of the Fracture resistance (N) and flexural strength (MPa) of tested groups. The horizontal line at 63.2% probability of failure was added for comparison between the groups. Air: Alert composite, Exf: Ever X Flow-, and FZ3: Nanocomposite

Discussion

The restoration of endodontically treated teeth requires special attention to restoring their function and aesthetics (Baba et al. 2017). The most common sequelae of restoring endodontically treated teeth are fractures either vertical or cuspal fractures. The incidence of these fractures in many types of research may range from 13 to 15% (Toure et al. 2011; Mehta and Millar 2008) and in some research it reached up to 28% (Chen et al. 2008). Premolars especially with endodontic MOD cavities are more susceptible to cuspal fracture after cusp deflection during function. It was noticed that the loss of the marginal walls makes these teeth severely prone to fracture (Wu et al. 2004; Belli et al. 2006). Larson et al. reported

that occlusal preparation decreases the fracture strength of the tooth by about 14–44%, while MOD preparation decreases the fracture strength by about 20–63% (Soares et al. 2008). For these reasons, premolar teeth with MOD cavities were included in the present study to represent the worst scenario as MOD cavities will decrease the fracture resistance.

Fracture resistance is the most examined for the invitro studies for the evaluation of the strength of different materials. Many factors can affect the result of the studies concerning fracture resistance. That includes the method of tooth mounting, type of load application device, and crosshead speed (Al-Makramani et al. 2013). Fracture resistance was tested using a universal testing machine

with the help of a 6 mm stainless steel sphere by applying an axial compressive load directed toward the center of the occlusal surface. Several studies have claimed that the use of stainless steel spheres is optimum for use as it creates a uniform contact with both functional and nonfunctional cusps, in this study a stainless-steel bar with a 3.8 mm diameter ball end was used to contact both buccal, palatal cusp ridges, and the restoration's center in premolar teeth with equal distance (Zarow et al. 2020). Moreover, the same idea of three-point contact was used in the current study. The load applied was in the direction of the long axis, this was by numerous studies that have used the same direction to test the fracture resistance of restorative systems (Belli et al. 2006; Nasr and Fawzy 2017).

As a result of the marked improvement in the physical and esthetic properties of advanced restorations, the resin composite becomes one of the primary choices for direct restoration. Moreover, it is not advocated for use in large restorations as its unable to reinforce the weekend tooth structure (Badakar et al. 2011). Accordingly, nano-filled composites were developed by incorporating nanometer particles into the resin matrix (Jain and Wadkar 2015). As compared to conventional composites, nanocomposites improved the abrasion resistance, they also own a greater modulus of elasticity, and higher impact, flexural, and tensile strengths (Sachdeva et al. 2015). Fiber reinforcement resin composite is a group of materials that have high strength and toughness that allow them to be used in many applications in dentistry. It was claimed that these materials can prevent crack propagation. The short fiber-reinforced resin composite was introduced to dentistry as bulk dentine replacement material. It is composed of short E-glass fiber and inorganic fillers embedded in a polymer network matrix (bis-GMA, TEGDMA, and PMMA) (Garoushi et al. 2006).

Two different commercially available short fiber reinforced composites that are especially used in stress-bearing areas were evaluated in this study regarding their fracture resistance and flexural strength when applied in MOD cavities of endodontically treated teeth. In this study, the use of conventional composite overlaying Ever X flow was recommended by many authors, especially in large cavities with high stress-bearing areas. This biomimetic technique allows for more stress distribution and decreasing in polymerization shrinkage (Goda and Abogabal 2020; Tsujimoto et al. 2016).

Although, ANOVA tests are the most popular, however, reporting the results using Weibull analysis was recommended by many authors (Armstrong et al. 2017; Abdou et al. 2021). Weibull analysis can help determine the reliability of the tested parameter. For comparison between tested groups, some authors report the comparison at

"a" (Abdou et al. 2021) and others report at P10. Both are common methods of reporting and recommended to report the comparison of both parameters based on ISO standards (Tichy et al. 2020).

Several studies evaluated the effect of fiber-reinforced resin composite on the teeth strength, their results varied according to the type of fiber used, the technique of fiber insertion, and the test method applied (Fennis et al. 2005; Garoushi et al. 2013; Shafiei et al. 2014; Abouelleil et al. 2015; Garoushi et al. 2015). According to the results obtained from this current study, EverX flow had statistically the highest fracture resistance and flexural strength when compared to the other types of resin composite. On the other hand, Alert has more fracture resistance and flexural strength when compared to nano-filled resin composite even though the difference between them was statistically insignificant. These findings were following that of previous studies which show superior fracture resistance and flexural strength owing to short fiber-reinforced resin composite when compared to bulk fill resin composites or conventional composites (Garoushi et al. 2013; Abouelleil et al. 2015). The superior effect of EverX flow may be explained by its unique structure which contains E-glass fiber fillers that approximately equal to or slightly exceed the critical fiber length, this results in random orientation of these shot fibers inside the restorations. This randomly oriented short fiber in addition to the low density of the polymer matrix increases the ability of the restoration to resist fracture and also improves the transformation of stresses from the matrix to the fibers (Moosavi et al. 2012; Garoushi et al. 2015). On the other hand, adequate fiber length enhances proper adhesion between the fiber and the polymer matrix, this good adhesion with minimal voids improves the strength of the composite (Lassila et al. 2020). In addition, several authors claimed that short fiber-reinforced composite can react more naturally as it had more dentine-like fracture resistance which is the case in EverX flow (Garoushi et al. 2015; Fennis et al. 2005).

Other possible explanations could be the chemical bond between the resin and the fiber and also the properties of the fiber itself. The presence of leno weave in the fiber of the EverX flow acts as a barrier to crack propagation and thus increases the crack resistance and decreases the shrinkage stress (Mohan et al. 2019). Each fiber act separately as a crack stopper by absorbing the stress forces applied to the matrix and thus preserving the structural integrity of the tooth and decreasing the failure mode. On the other hand, part of the composite was replaced by the fiber which result in a diminishing of the volumetric contraction of the resin (Ayad et al. 2010).

On contrary, Fráter et al. (2014) demonstrated that there was no statistical difference in fracture toughness

between short fiber-reinforced resin composite and conventional composite material. Moreover, Atalay et al. (2016) stated that EverX was not significantly different from other tested restorative materials in fracture resistance. This contradiction may be accredited to the difference in material application, the difference in sample preparation, or may be related to the variance in the type of adhesive used.

According to the result of this study, the EverX flow resin composite shows higher fracture resistance in contrast to the Alert. The fiber length and diameter have a great effect on the distribution of forces among the polymer matrix, EverX flow has a fiber length of $200-300~\mu m$ and a diameter of 6 μm while Alert has a fiber length of $20-60~\mu m$ and diameter of 7 μm . Thus, EverX flow has a longer fiber length with a shorter diameter that approximates the range of the recorded critical fiber length. This allows the EverX flow to act as a biomimetic restoration that reproduces a similar layer as in the natural teeth (Lassila et al. 2020).

Flexural strength resembles the flexibility of the materials, which means that the high flexural strength of the material indicates brittle properties and high hardness (El-Shekeil et al. 2012). According to the result of this study nanocomposites show the lowest flexural strength. On the other hand, EverX flow was statistically higher in flexural strength than Nanocomposite. Benkhelladi et al. 2020 stated that the addition of fibers increased the flexural properties of the composites, they also noticed that the incorporation of more than 40 wt% fiber in the composite improves its mechanical properties. All the 3 tested composite types have fiber content that exceeds the recommended fiber percentage. However, the low flexural strength of nanocomposite may be attributed to the size of the fiber incorporated in its matrix, claiming to its nano size, these nanofibers started to form bundles or clusters that create mechanical weak points in the composite and led to a decrease in conversion rate and thus lower the mechanical properties of the material (Badakar et al. 2011; Vidotti et al. 2015).

The superior strength outcome of the EverX flow in comparison to Alert and nanocomposite may be appertaining to the size and weight of short fibers incorporated in the matrix that impair the bonding resistance. Accordingly, the null hypothesis was rejected as EverX flow short-fiber-reinforced resin composite resulted in improved fracture resistance of MOD restored cavities.

Conclusions

Based on the finding of the present study, it can be concluded that the commercially available short-fiber reinforcement composite has adequate fracture resistance and flexural strength which promote it to be a suitable material for the restoration of endodontically treated teeth with a large cavities.

Recommendations

Further studies must be done with the inclusion of thermocycling and water storage tests to further mimic the in vivo conditions in order to evaluate the plasticizing effect of these tests on different types of fiber-reinforced composite.

Abbreviations

MOD: Mesio-Occluso-Distal cavities; ETT: Endodontically treated teeth; CEJ: Cementoenamel junction; Alr: Alert fiber-reinforced resin composite; Exf: Ever X Fiber-reinforced composite; FZ3: Filtek Z350 nano-filled resin composite.

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Author contributions

WG: Conceptualization, Methodology, Investigation, Writing-original draft. AA: Formal analysis, Visualization. GS: Conceptualization, Investigation, Writing-original draft. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The raw data required to reproduce these findings are available upon reasonable request from the corresponding author. The processed data required to reproduce these findings are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The current research was organized by the Ethics of the World Medical Association, following the assumption conveyed in the Declaration of Helsinki. The approval of this research was earned from the local Ethical Committee of the National Research Centre, Cairo, Egypt with approval number (3435062021).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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