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ORIGINAL RESEARCH

Short fiber reinforced composite on fracture strength of immature permanent anterior teeth with simulated regenerative endodontic procedures: an *in vitro* study

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Abstract

The aim of the present study was to evaluate the effect of short fiber reinforced composite on the fracture strength of anterior immature teeth treated with regenerative endodontic procedures. A total of 120 permanent maxillary central incisors were selected, and root lengths were standardized. Except for the positive control group (n = 20), the root canals were instrumented to simulate immature teeth with incomplete root development, and the regenerative endodontic procedure was performed. Twenty instrumented teeth acted as negative controls (n = 20), and the remaining 80 teeth were randomly divided into 4 groups according to the chosen coronal restoration material: bulk fill, short fiber reinforced composite (SFRC), polyethylene fiber (Ribbond Ultra), and flowable composite resin. Each specimen was then subjected to fracture testing using a universal testing machine (AGS-X, Shimadzu, Japan). The load to fracture was recorded. Data were subjected to statistical analysis using analysis of variance and the Tukey Honestly Significant Difference test. A significant difference was detected between the groups ($p < 0.05$), with the positive control group showing the highest mean fracture strength. The SFRC group had significantly higher values than the bulk fill, polyethylene fiber, flowable composite resin and negative control groups. In conclusion, SFRC has a relatively high fracture strength compared to other materials used in regenerative endodontic procedures. The use of SFRC enhanced the fracture strength of immature permanent teeth.

Keywords

Fracture strength; Permanent; Regenerative endodontics

1. Introduction

Major challenges associated with nonvital immature tooth pulp treatments include thin root walls and open apexes. Conventional treatment modalities used in immature teeth can lead to abnormal root morphology, weakened dentin and root fractures [1, 2]. In addition, these teeth are vulnerable to fracture due to the large access cavity required to treat these cases, especially when the defective crown-to-root ratio, long-term apexification treatments, or one-step apical barrier techniques are selected [3]. Thus, regenerative endodontic procedures (REPs) are widely used when treating immature teeth. Regenerative pulp treatment modalities may also provide promising results, as new pulp tissue can develop from undifferentiated cells [4]. The current literature discusses cell homing and root canal disinfection strategies and ways to achieve more efficient and economically successful treatments. Other main topics include long-term follow-up and the causes of failure in teeth treated with REPs [1, 5–7]. At present, no consensus has been reached to define the failure of regenerative endodontics cases [1, 8–10].

One of the concerns regarding REPs is the subsequent fracture of teeth in the cervical areas after treatments [11]. The aim of REPs is to induce further root development and strengthen the tooth; however, the cervical area does not develop further following this procedure. The cervical regions are particularly prone to fractures because of functional stresses [12]. Although REPs are clinically advanced procedures, the root walls of the treated teeth (especially the cervical areas) require critical strengthening to avoid fracture [13, 14]. Cervical fractures have been observed in approximately three-quarters of immature teeth with minimal root development during endodontic treatment or secondary trauma. Decision-making and management of immature teeth therefore present a particular challenge for clinicians due to the noticeably high incidence of fracture [15].

Although the biological goals of REPs can be achieved, the treatment outcome is not always predictable, and strengthening the cervical area is the clinician’s responsibility [16]. Several coronal treatment options are currently used for endodontically treated teeth, but the main goal is to conserve the remaining dental structure, especially the cervical region [17]. Therefore, the choice of restoration material is important.

Polyethylene fibers are a restoration material containing a bondable reinforcement consisting of ultra-high strength polyethylene fibers. The use of these fibers with a bonding agent and a flowable composite can aid in absorbing stresses by providing a lower elastic modulus [18]. Endodontically treated teeth can also be restored with short fiber reinforced composite (SFRC), a resin-based material that incorporates both polyethylene and glass fibers and mimics the dentin structure to absorb stress in risky areas [19]. The development of SFRC technology has greatly increased the use of composite resin materials in endodontics. The formation of fiber-reinforced composite resins creates a whole structure from the polymer matrix and the fibers, so that the stresses that occur in the matrix structure are transmitted to the fibers, thereby preventing the development of fractures that may occur in the restoration or in the tooth [20].

Studies that have examined the fracture resistance of endodontically treated teeth filled with Biodentine and mineral trioxide aggregate (MTA) as cervical barriers and restored with various restorative materials are available in the literature [21, 22]. Other studies have reported how different coronal restorations affect the fracture strength in REP-treated posterior teeth [22]. A recent study compared the clinical success of SFRC and glass-ceramic endocrowns and found that both showed similar success [23]. Nevertheless, the lack of knowledge regarding fracture strength when managing immature teeth with regenerative endodontic protocols leads to unpredictable and questionable outcomes, and clinicians are reluctant to incorporate REPs into their everyday practice until more evidence for good biological outcomes is available [14, 15]. Data are particularly lacking for SFRCs and their use in the coronal restoration of teeth treated with REPs.

The aim of the present study was to evaluate the use of SFRC for improving the fracture strength of anterior immature permanent teeth treated with REPs. The null hypothesis of the present study was that no differences between SFRC group and the other treatment groups.

2. Materials and methods

2.1 Sample size calculation

The sample size was calculated G*Power (latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) as 13 for per six groups, according to the data of a previous study by Plotino *et al.* [24]. A sample size of 120 was used to ensure reliable results for this *in vitro* study.

The teeth selected for inclusion in the present study were extracted for periodontal reasons, without caries, with one root and one canal. The mean dimensions of the 120 maxillary central incisors were 6 $\pm$ 0.5 mm in the buccolingual direction and 7 $\pm$ 0.5 mm in the mesiodistal direction. Root surfaces were examined at $\times$20 magnification with a stereomicroscope (Leica microsystems, Wetzlar, Germany) for cracks or fractures. Teeth with any fractures or cracks were excluded from the study.

2.2 Simulation of an immature tooth

The teeth were kept in 0.1% thymol solution until the study time. Root lengths were standardized by cutting each tooth with a precision of 12 $\pm$ 1 mm from the cementoenamel junction to the apical root tips using a slow-speed diamond precision saw under water cooling (Isomet 1000; Buehler, Lake Bluff, IL, USA). A sample of 20 teeth was reserved as a negative control group.

An immature root apex was simulated by creating canal entrance cavities with a round diamond bur of the same size and grain with water cooling. The root canals were enlarged up to the K-type No. 60 file (Dentsply Sirona, Tulsa, OK, USA). Reciproc files were used when preparing the canals (VDW, Munich, Germany) to a #50. Canals #1 to #6 were instrumented with Pieso Reamers (Dentsply Maillefer, USA) to simulate immature teeth with incomplete root development, using a #6 Pieso Reamer (1 mm) from the apical region when the instrumentation process had been completed.

The REP guidelines of the American Association of Endodontists (AAE), updated in 2021, were followed. Root canals were irrigated using 2 mL of 1.5% sodium hypochlorite after each instrument. After preparation, irrigation was provided with 17% EDTA solution, followed by 5 mL of distilled water, and then the root canals were dried using paper points. Metronidazole (500 mg; Flagyl; Sanofi, Istanbul, Turkey) and ciprofloxacin (500 mg film tablet; Cipro; Biofarma, Istanbul, Turkey) were mixed at a ratio of 1:1 and placed into the root canals using a size 40 lentulo (Dentsply Sirona, Tulsa, OK, USA) to simulate the disinfection procedure for REP. The canals were temporarily closed with cotton pellets, and the tooth coronal access cavities were covered with glass ionomer (Ketac Molar Easy Mix 3M ESPE, Germany). All samples were stored in an oven set at 37 °C (Core incubator EN 120, Istanbul, Turkey) and 100% humidity for 4 weeks.

After 4 weeks, the double antibiotic paste was removed by rinsing with 15 mL of 17% EDTA using the conventional needle irrigation technique, and paper points were used to dry the root canals.

MTA (3 mm thick; Pro Root MTA, Dentsply Maillefer, Ballaigues, Switzerland) was mixed according to the manufacturer's instructions and placed in the coronal third of the canal of all samples except for the negative control group. A moist cotton pellet was placed on the MTA and we also utilized a collagen material called Collaplug (Zimmer Dental, Carlsbad, CA) to limit the depth of MTA. Interim restoration as glass ionomer cement (Ketac Molar Easy Mix 3M ESPE, Germany has been used in the current study). The teeth were wrapped in gauze soaked in phosphate buffered saline (pH = 7.4) and incubated at 37 °C for 4 days.

2.3 Coronal restoration procedure

The test group teeth (the remaining 80 teeth) were subjected to selective enamel etching with 35% phosphoric acid (Scotchbond Etchant, 3 M ESPE, St Paul, MN, USA). Clearfil^{TM} SE Bond (Kuraray, Okayama, Japan) was then used in the cavities according to the manufacturer's instructions. The 80 teeth were then randomly divided into 4 groups according to the choice of coronal restoration material to be used.

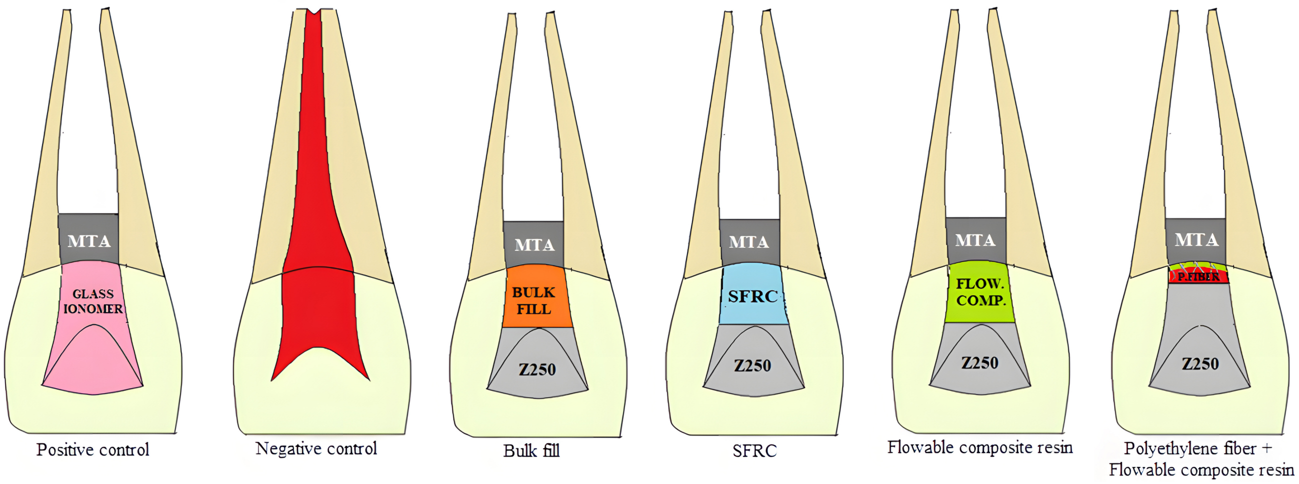

FIGURE 1. The schematic appearance of study groups. The final distributions of the teeth in the control and test groups. MTA: mineral trioxide aggregate; SFRC: short fiber reinforced composite.

The final distributions of the teeth in the control and test groups in the present study were as follows (Fig. 1):

1. Positive control group (n = 20): Immature tooth simulation was performed in this group. REPs were performed. This group was restored only with glass ionomer cement (n = 20).

2. Negative control group (n = 20): Sound teeth with only the apical tip cut (to standardize root lengths). No immature tooth simulation, REP or restoration was performed.

3. Bulk fill group (n = 20): The cavity was filled with a bulk-fill composite resin (3 M ESPE) and cured for 20 seconds. Polymerization was achieved for a period of 1 min. The restoration was completed with conventional composite resin (3M ESPE Filtek Z250).

4. SFRC group (n = 20): The cavity was filled with short fiber-reinforced composite resin (EverX Posterior GC, Tokyo, Japan) for 20 seconds. Polymerization was achieved for a period of 1 min. The restoration was completed (3M ESPE Filtek Z250).

5. Flowable composite resin group (n = 20): Flowable Restorative Refill was applied to the cavity floor and polymerized for 20 seconds. The restoration was completed (3M ESPE Filtek Z250).

6. Polyethylene fiber group (n = 20): A polyethylene fiber piece was cut using sterile surgical scissors to a length of 1 mm and a width of 1 mm and soaked with Clearfil^{TM} SE Bond adhesive. A thin layer of 3M^{TM} Filtek^{TM} Supreme flowable composite resin was used to coat the cavity walls. The polyethylene fiber (Ribbond Ultra Inc., Seattle, WA, USA) piece wetted with the bond adhesive was placed on the bottom of the cavity where the flowable composite resin had been applied and left for 20 seconds. Polymerization was achieved with a light device (3M ESPE Elipar S10) for 20 s. The remaining part of the cavity was restored (3M ESPE Filtek Z250).

2.4 Periodontal ligament simulation and fracture strength test

A thin layer of silicone impression material was used to cover the surfaces of the tooth roots, due to the simulation of the periodontal ligament (Zhermack, Rovigo, Italy). Acrylic resin was used to simulate the space between the root surface and the bone crest. The resin was vertically implanted approximately 2 mm below the cementoenamel junction.

A universal test device (Autograph, Precision Universal Tester, AGS-X, Shimadzu, Japan) was used for the fracture test. The stainless steel spherical tip of the test device was placed 3 mm above the cementoenamel junction line on the palatal surface and adjusted to create an angle of 135 degrees between the upper part of the tooth and the long axis of the tooth. A force was applied at a speed of 1 mm/min. When the tooth broke, the test device was stopped, and the values on the screen were recorded in Newtons. Fractures were more prevalent in the cementoenamel junction CEJ or root area near the CEJ, which means unrestorable fractures (Fig. 2).

2.5 Statistical analysis

The data were analyzed using the SPSS software (version 21.0; IBM Corp., Armonk, NY, USA) using descriptive and analytical statistics. The data were tabulated and evaluated with the Kolmogorov-Smirnov and Shapiro-Wilks tests, which showed a normal distribution. The control and test groups were compared using analysis of variance (ANOVA), and the Tukey Honestly Significant Difference (HDS) test ($p < 0.05$), at a 95% confidence interval (CI) for pairwise comparisons.

3. Results

The mean fracture strength values of all sample groups are presented in Table 1, which shows a statistically significant difference in fracture strength among the groups ($p < 0.05$). The mean fracture strength test values were significantly higher for the negative control group (700.02 $\pm$ 15.67 N) than for all the

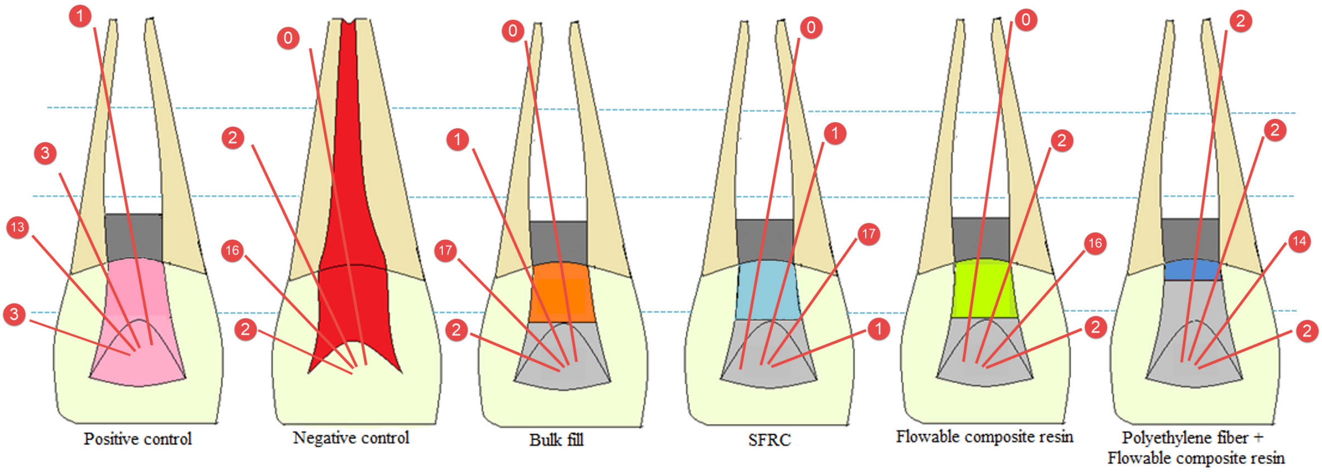

FIGURE 2. Schematic illustration of fracture locations of all the groups. SFRC: short fiber reinforced composite.

TABLE 1. The values of the fracture strength of all the sample groups.

	Fracture Strength (N)			
	Minimum	Maximum	M ± SD	95% CI
Positive control	657.69	723.99	700.02 ± 15.67*	692.68–707.36
SFRC	632.48	712.64	674.33 ± 18.31*	665.76–682.90
Bulk fill	402.76	434.84	415.51 ± 9.32*	411.14–419.87
Polyethylene fiber + Flowable composite resin	561.18	634.68	605.12 ± 18.58*	596.42–613.81
Flowable composite resin	432.76	487.67	466.73 ± 14.39*	459.99–473.47
Negative control	176.46	214.76	198.83 ± 11.49*	193.45–204.20
p			0.001*	

*One-way ANOVA Test. *p < 0.05. *in the columns indicate statistically significant differences between groups (CI: 95%). M: Median; SD: Standard Deviation; SFRC: short fiber reinforced composite; CI: Confidence Interval.*

other groups ($p < 0.05$). The negative control group and the SFRC group values were similar ($p < 0.05$). The values were also significantly higher for the SFRC group than for the bulk fill, polyethylene fiber, flowable composite resin or positive control groups ($p < 0.05$). The values were significantly higher for the polyethylene fiber group than for the bulk fill, flowable composite resin or positive control groups ($p < 0.05$). The values were significantly higher for the flowable composite resin group than for the bulk fill or positive control groups ($p < 0.05$). The mean fracture strength was significantly higher for the bulk fill group than for the positive control group ($p < 0.05$) (see Fig. 3).

4. Discussion

REPs, unlike traditional apexification techniques, can lessen the risk of root fractures caused by thin roots. However, the cervical regions are prone to fractures because of functional stresses after REPs [13, 25]. Limited data are available in the literature regarding this issue [21, 22]. Thus, the aim of the present study was to evaluate the efficacy of SFRC by comparing the fracture strength of anterior teeth treated with REPs.

Among the test groups, the highest fracture strength values were observed in the SFRC group. Because statistically significant differences were detected among the groups, the null hypothesis was rejected. The differences between the present study and previous studies could be attributed to the use of different methodologies. One *in vitro* study stated that SFRC resin had poorer fracture strength values than polyethylene fiber [21]. Another study that tested premolar teeth stated that the SFRC resin group exhibited fracture strength values higher than the other groups. SFRC resins have matrix structures consisting of cross-linked bisphenol A-glycidyl methacrylate (bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) monomers, a combination that results in a polymer network during polymerization and provides good bonding and resistance to fractures [26, 27].

The use of fiber reinforcement is justified in part for internal fortification of the structurally compromised tooth and in part to avoid fractures. The length of the fibers, their orientation, their position, their adhesion to the polymer matrix, and their impregnation into the resin all influence the effectiveness of the fiber reinforcement. Stress transfer from the polymer matrix to the fibers underlies the reinforcing effect of the fiber fillers, but each fiber also serves as a crack-stopper per se. Transfer of the stress from the polymer matrix to the fibers is crucial, and SFRC material is produced for the purpose of using it as a bulk base in areas with high stress for the restoration of vital and nonvital teeth [28]. SFRC

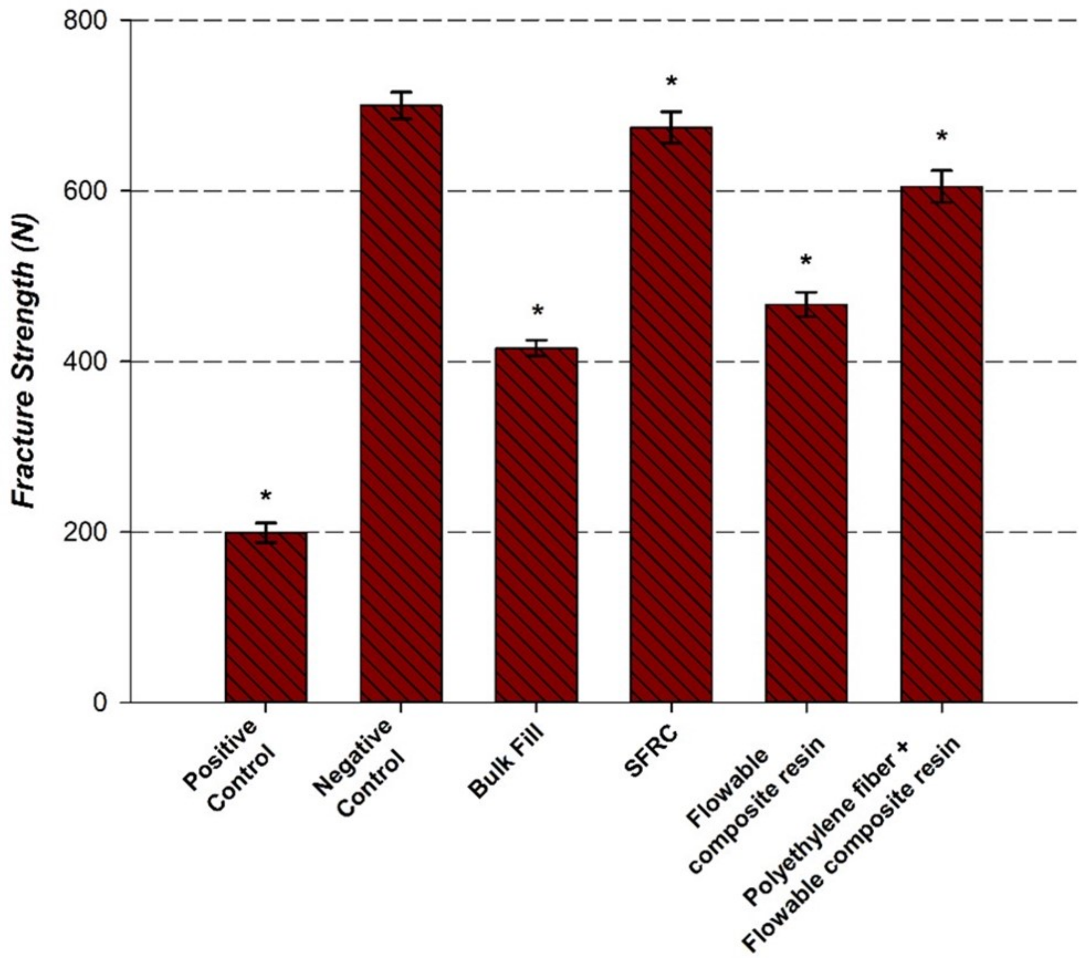

FIGURE 3. The values of the fracture strength of all the sample groups. *shows a statistically significant difference in fracture strength among the groups. SFRC: short fiber reinforced composite.

resin and glass-ceramic endocrowns showed similar success in a previous clinical study [23]. The number of *in vitro* studies on the topic is insufficient; however, this previous clinical investigation emphasized the use of SFRC material because it is simple to use and requires less processing than other materials. The findings of the current study agreed with previous work and confirmed the suitability of SFRC material for REP purposes and supported its use for reinforcement of the dental structure and improvement of fracture resistance when applied according to biomimetic principles.

The effect of polyethylene fibers on the fracture strength of teeth that have undergone endodontic treatment has been examined in several studies [16, 26]. Polyethylene fibers in the restoration of endodontically treated teeth increased the fracture strength of the teeth significantly [29]. In the current study, the fracture strength values were higher for the polyethylene fiber group than for the other experimental groups, except for the SFRC group. On the contrary, Balkaya *et al.* [21] found that the fracture strength was significantly higher for polyethylene fibers than for SFRC. Therefore, polyethylene fibers are also considered a promising option for strengthening the cervical region of immature teeth. In one study, inserting a piece of polyethylene fiber in the buccal to lingual direction made molar teeth with mesio-occlusal-distal (MOD) cavities more resistant to fractures [29].

In the present study, a piece of polyethylene fiber, wetted with a bonding adhesive, was placed on the bottom of the cavity. Many different strategies are possible for placing fibers; therefore, more studies need to be conducted to determine the most effective placement method. This polyethylene fiber material is made with a cross-locked, multi-directional, leno-weave style. When used in teeth that have suffered excessive damage, the flexibility of the polyethylene fibers acts on the stress dynamics and transmits incoming forces quite effectively [19, 30].

Any discussion of the positive effects of fiber-reinforced materials on fracture strength observed in the present study should also emphasize that the use of bulk-fill composite resin did not significantly enhance the strengthening effect compared to the polyethylene fiber, SFRC or flowable composite resin groups. The bulk-fill group had the lowest fracture strength in the study. The average fracture strength was significantly higher for the flowable composite resin group than for the bulk-fill or negative control groups. Previous simulations of the immature tooth method [15, 31] randomly categorized the teeth into groups based on structural differences, such as dentin, enamel and cementum thicknesses in the teeth of different individuals to prevent these differences from affecting the results [32]. In the present study, standardization of the periodontal ligament simulation was also considered important, so the root circumference was covered with a thin layer of silicone impression material. Furthermore, applying a load at a 135° angle to the cingulum, as performed in previous studies, was similarly implemented in the present study [15, 31]. The application of all these standardization methods together was one of the strengths of the current study.

This study's methodology had drawbacks and limitations especially for teeth under revascularization procedure. In addition, the challenges associated with finding the human immature teeth and the potential use of simulated immature teeth have been discussed.

After REP, the root thickness may be increased to improve fracture resistance. The problem is the thin part of the cervical part, but it is questionable whether the method proposed in this study can affect the resistance of cervical fracture. This is because the cervical part was still restored using MTA. As part of the regenerative endodontic procedure, we utilized bioactive MTA in the cervical portion of the root and aimed to reinforce the cervical region with different restorative materials applied to the crown. Our objective was to enhance the strength of the cervical region using different restorative materials. In future studies, it may be possible to compare different biocompatible materials and MTA for supporting cervical region. We preferred to use glass ionomer cement as a non-reinforcing restorative material in the coronal region due to the challenges encountered in the applicability of fracture testing in the positive control group. This can be considered as a limitation of the study and may have also influenced the results.

Furthermore, the fact that not all fractures occurred entirely at the cervical level in the fracture test can be considered as limitations of the study. Therefore, future studies are needed that using different placement method of fibers. To more accurately determine the effectiveness of various treatment modalities for immature teeth, the results of this study should be confirmed in clinical trials with large sample numbers and long-term follow-up. Within the limitations of this study, the results exerted that SFRCs strength the immature teeth when they are used after regenerative endodontic procedures.

5. Conclusions

SFRCs have a relatively high fracture strength when compared to other materials in teeth treated with regenerative endodontics. Long-term follow-up and clinical studies with larger sample sizes are needed to corroborate the findings of this investigation and to provide a more accurate determination of the effectiveness of various treatment modalities for immature teeth.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ŞTK—designed the research study, wrote the manuscript. İK—performed the research, analyzed the data. HA—provided help and advice for designing the study, helped to proofreading and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was obtained from the Health Sciences University Hamidiye Scientific Resarch Ethics Committee (approval number: 21/773). All patients filled out a consent participate before the extraction of the tooth samples (Patients aged under 16, the informed consent were signed by their guardians).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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