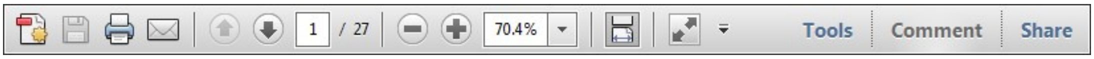
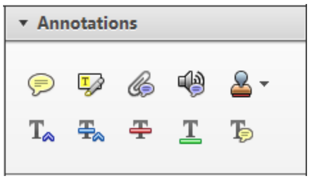
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

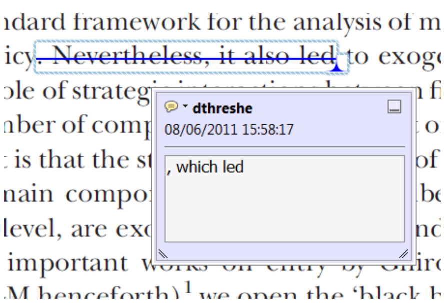


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

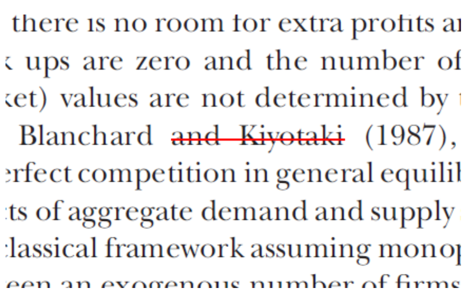


2. Strikethrough (Del) Tool – for deleting text.

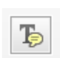
Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.



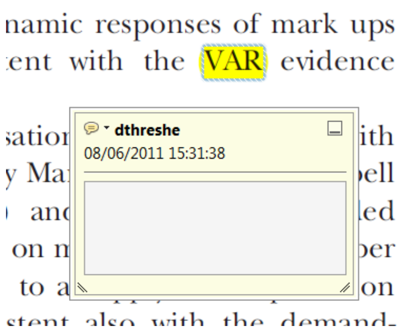
3. Add note to text Tool – for highlighting a section to be changed to bold or italic.



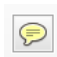
Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.



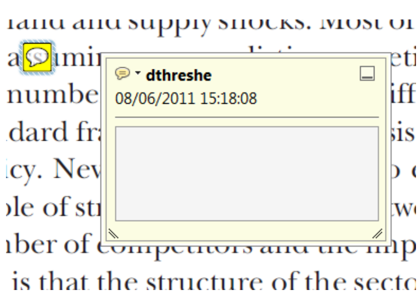
4. Add sticky note Tool – for making notes at specific points in the text.



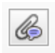
Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



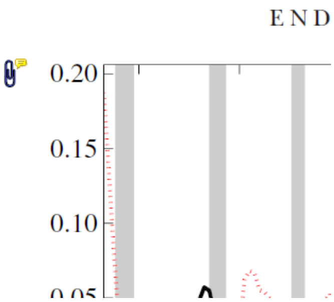
5. Attach File Tool – for inserting large amounts of text or replacement figures.



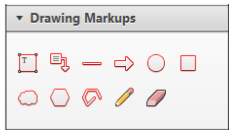
Inserts an icon linking to the attached file in the appropriate place in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

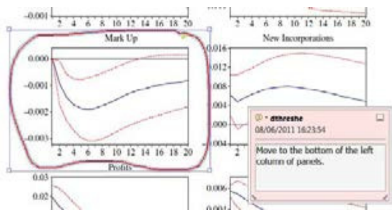


6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.
Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



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The effect of various backfilling techniques on the fracture resistance of simulated immature teeth performed apical plug with Biodentine

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Aim. To evaluate the fracture resistance of simulated immature teeth that had been backfilled using different materials after using Biodentine as the apical plug material.

Design. Seventy-five single-rooted teeth were divided into five groups (n = 15). The 15 teeth in group 1 served as a negative control group and received no treatment. The remaining 60 teeth were instrumented to a #6 Peeso reamer to obtain a standard internal diameter of 1.5 mm. The apical 4 mm of 60 teeth was filled with Biodentine. The backfilling was then performed on each group as follows: group 2 – no backfilling (positive control), group 3 – gutta-percha, group 4 – fiber post, and group 5 – Biodentine. Specimens were then subjected to fracture testing. The force required to fracture each specimen was recorded, and the data were statistically analyzed.

Results. The mean fracture values of groups 1 and 4 were significantly higher than groups 2, 3, and 5 ($P < 0.05$). The values of groups 3 and 5 were significantly higher than group 2 ($P < 0.05$).

Conclusions. The backfilling with fiber post after an apical Biodentine plug provided the highest fracture resistance among all experimental groups.

Introduction

Pulp necrosis that occurs in the permanent dentition due to trauma or caries might cause the termination of root formation in developing teeth[1]. Pulpal necrosis of an immature tooth will necessitate endodontic treatment that can pose both endodontic and restorative challenges because of incomplete closure of the apex and thin dentinal walls[2,3]. Thin dentinal walls predispose these teeth to a higher incidence of root fractures[4].

Traditionally, the treatment approach has been to use calcium hydroxide (CH) to induce apexification after disinfection of the root canals in the conventional manner. However, apexification using CH is subject to variable treatment time, unpredictable of formation of an apical seal, difficult patient follow-up, and delayed treatment[3]. Additionally, it has been shown that CH, on prolonged contact with dentin, adversely affects strength and fracture resistance[5]. Other methods for treating teeth with an immature apex are available as an alternative to CH therapy: mineral trioxide aggregate (MTA) apexification and pulp revascularization[6]. Revascularization has shown great potential for clinical success[7–9] but has yet to be evaluated over the long term and may not be successful in every case. Garcia-Godoy and Murray[10] stated that regenerative endodontic treatment is not recommended for patients younger than 7 years or older than 16 years, or those with a systemic disease that can impair healing. When revascularization is not an option or has not been successful, other procedures such as apexification including an apical plug with MTA have been carried out[11].

In apexification procedures, a significant concern is determining which material to use for filling the remaining canal space after applying an apical plug. There are reports that assess the effectiveness of various filling methodologies, including fiber post, gutta-percha,

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and composite resin on the reinforcement capacity of immature teeth after an apical MTA plug has been placed to induce apexification[12,13]. However, a consensus on this issue has not been reached.

Recently, a new calcium silicate-based material, Biodentine (Septodont, Saint Maur-des Fausseś, France), was introduced. According to the manufacturer, Biodentine contains tricalcium silicate, calcium carbonate, and zirconium oxide, and a water-based liquid that contains calcium chloride as the setting accelerator and a water-reducing agent[14]. It is a fast-setting (approximately 10–12 min) calcium silicate-based restorative material recommended for use as a dentin substitute and which can be used in apexification, internal/external resorption, pulp capping, furcation perforation, and retrograde surgical filling[15,16]. Additionally, the use of Biodentine to obturate the entire root canal space was evaluated in a recent study, and it was concluded that the bond strength of Biodentine on the root canal walls was higher those of gutta-percha/sealer combination[17].

The purpose of this study was to evaluate the fracture resistance of simulated immature teeth restored with various backfilling methodologies including Biodentine, a fiber post, and gutta-percha/sealer combination after an apical Biodentine plug placement.

Materials and methods

Ethical approval and sample size calculation

The protocol of this study was approved by the Research Ethics Committee of Erciyes University, Kayseri, Turkey (number of the document:196/2014). Results of power calculation based on research of Wilkinson *et al.*[2] have revealed that the sample size for each group is minimum 15. This value has been determined by projecting the power as 0.92, effect size = 0.712, and significant level as $a = 0.05$.

Tooth preparation

Seventy-five extracted human maxillary central incisor teeth were used in the study and were stored in 0.1% thymol until the beginning of the experiment, but for no longer than 30 days after extraction. The teeth were examined under an operating microscope (Carl Zeiss OPMI PICO, Jena, Germany), and any roots with root caries, cracks, or fractures were excluded. Preoperative radiographs were taken in the facial-lingual and mesio-distal directions to confirm the presence of a single canal without previous root canal treatment, resorptions, or calcifications. The facial-lingual and mesial-distal dimensions of each tooth were measured using a digital caliper (Teknikel, Istanbul, Turkey) at the cemento-enamel junction (CEJ). All roots were of similar dimensions measuring 5.63 ± 0.5 mm facial-lingually and 6.37 ± 0.4 mm mesio-distally. Each tooth was sectioned to obtain a length of 8 mm above and 12 mm below the facial CEJ. Fifteen teeth were not instrumented and served as negative controls (*Group 1*). The remaining 60 teeth were prepared as follows: Endodontic access cavities were made using a round bur (Dentsply Maillefer, Tulsa, OK, USA) and an Endo Z bur (Dentsply Maillefer) in a high-speed handpiece. The root canals were prepared using the ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) to a size 50, 0.05 taper (F5). To achieve simulation of teeth with immature apices, Peeso reamers (Mani Inc., Tochigi, Japan) between #1 and #6 were introduced in the root canals and a #6 Peeso reamer was allowed to protrude 1 mm beyond the apex. The root canals were irrigated using 3 mL of 3% sodium hypochlorite after each instrument, and a final flush with 5 mL of 17% EDTA was carried out to remove the smear layer. Finally, the root canals were flushed with distilled water and dried using paper points (Dentsply Maillefer). To simulate clinical conditions, calcium hydroxide paste (UltraCal XS; Ultradent, South Jordan, UT) was placed in the canals using a 29-gauge NaviTip (Ultradent). The root canal accesses were then filled using a temporary filling material (Cavit, 3M ESPE, Seefeld, Germany), and the samples were kept for 1 week at 37°C under 100% humidity. After 1 week, the temporary filling materials were removed from the access cavities, and the calcium hydroxide was removed with passive activation of 5 mL of 2.5% NaOCl using a smooth ultrasonic file (size 15, .02 taper) (ESI instrument; EMS, Le Sentier, Switzerland) mounted in an ultrasonic device (EMS) at a power setting of 6 for 1 minute. The canals were then rinsed with 5 mL of 17% EDTA and 5 mL of distilled water and were dried with paper points. Biodentine (Septodont) liquid from a single-dose container was emptied into a powder-containing capsule and mixed for 30 s at 4000 rpm, according to manufacturer's instructions. Biodentine was then placed with a carrier under the operating microscope in the apical portion of the canal (4 mm) to create an apical plug. After the positioning of the apical Biodentine plug, it was adapted to the canal walls using a hand plugger (Sybron-Endo, Orange, CA, USA), in proportion to the apical gauge. The teeth were radiographed to verify the correct position of the Biodentine mixture. Subsequently, the samples were wrapped in wet gauze, placed in an incubator, and allowed to set for 12 min at 37°C with 100% humidity. The teeth in the control group and experimental groups were then treated as follows.

Group 2 – Positive control (n = 15). No obturation, other than the apical Biodentine plug, was performed. A cotton pellet was placed at a level just below the facial CEJ.

Group 3 – Backfill with gutta-percha (n = 15). AH Plus sealer (Dentsply Maillefer) was mixed according to manufacturer's instructions and applied to the canal walls using a lentulo spiral (Dentsply Maillefer). The canals were backfilled with gutta-percha using a BeeFill 2 in 1 obturation unit (VDW, Munich, Germany). The gutta-percha was vertically compacted with pluggers. Excess sealer was removed from the chamber with a dry cotton pellet.

Group 4 – Backfill with fiber post (n = 15). In this group, the post spaces were created using #2 Rely X post drills (3M ESPE) in the remaining unfilled portion of the root canal. Surfaces of the posts were cleaned with alcohol and dried with air. The root canals were rinsed with water using a syringe and gently dried with paper points. Rely X Unicem 2 self-adhesive resin cement (3M ESPE) was manipulated according to the manufacturer's instructions and inserted into the canal. A #2 Rely X fiber post (3M ESPE) was then seated in the root canal and inserted until the termination of the post space using finger pressure. The excess resin was immediately removed, and the light activation was performed for 40 s. The protruding coronal portion of the fiber post was trimmed, using a high-speed handpiece.

Group 5 – Backfill with Biodentine (n = 15). In this group, the canals were backfilled with Biodentine (Septodont). Biodentine was mixed as previously described and placed to a level just below the facial CEJ.

In groups 2–5, after backfilling procedures, the access openings were filled with nanocomposite (Filtek™ Z350 XT; 3M ESPE, St. Paul, MN, USA) and all specimens were stored in 100% humidity at 37°C for 2 weeks until fracture resistance testing. Figure 1 shows radiographs of representative samples of the groups.

Fracture testing

To simulate a periodontal membrane, the root surfaces were coated with a 0.2–0.3 mm thick layer of polyvinylsiloxane impression material (Coltene\Whaledent AG, Switzerland), before embedding the roots into acrylic resin. The specimens were then perpendicularly embed-

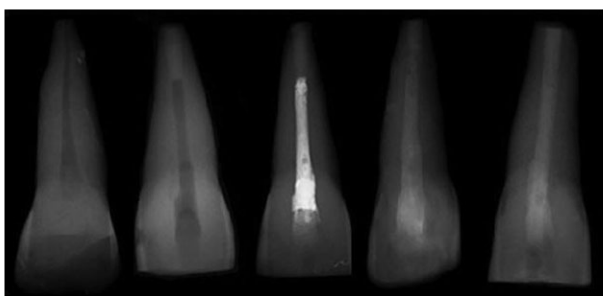

Fig. 1. Radiographs of representative samples of groups 1, Negative control; 2, Positive control; 3, Gutta-percha; 4, Fiber post; 5, Biodentine.

ded in copper rings (20 mm high and 20 mm diameter) and filled with self-curing acrylic resin (Imicryl, Konya, Turkey) leaving a gap of 2 mm between the top of the acrylic and the CEJ. This gap simulates the spacing found between the bone crest and the tooth[2]. A jig as described by de Melo *et al.*[18] was used to fix the cylinders at an angle of 45°. A universal testing machine (Instron Corp., Canton, MA, USA) was used for the strength test. A compressive load was applied from the palatal surface at a crosshead speed of 1 mm/min until fracture. The fracture moment was determined when a sudden drop in force occurred as observed on the testing machine display. The maximum force required to fracture each specimen was recorded in Newtons. The Kolmogorov-Smirnov statistical test for normality revealed a normal data distribution. The data were statistically analyzed using a one-way analysis of variance (ANOVA) with Tukey's *post hoc* test for multiple comparisons. The level of significance was set at $P = 0.05$.

The fracture patterns were evaluated using a modified classification system based on the classification system proposed by Santos-Filho *et al.*[19]: (a) fracture above CEJ, (b) fracture at CEJ level (c) mid-root fracture, (d) apical root fracture, and (e) vertical root fracture.

Results

The mean values and their respective standard deviations of the force required to fracture the roots are presented in Table 1. The results of the one-way ANOVA test indicated a significant difference existed between the groups ($P = 0.000$). The negative control group exhibited the highest resistance mean values to fracture, compared to all groups ($P = 0.000$) but fiber post group ($P = 0.765$). Gutta-percha and Biodentine groups presented the second highest mean values but with any significant difference between each other ($P = 0.998$). Positive control group displayed the lowest value compared to all groups ($P = 0.000$). All teeth fractured horizontally or obliquely at various levels of the cervical area of the root. The teeth in the fiber post group generally fractured above the CEJ; however, teeth in the other groups had fractures at the CEJ or mid-root fracture. Apical root fracture and vertical root fracture did not occur in any groups.

Table 1. Mean fracture values for all groups measured in Newtons along with their standard deviations.

Groups	*n*	Mean forces (*N*) ± Standard deviation
1. Negative control	15	1039.00 ± 202.18^{a}
2. Positive control	15	659.80 ± 101.93^{b}
3. Backfilling with gutta-percha	15	824.13 ± 114.65^{c}
4. Backfilling with fiber post	15	979.97 ± 104.05^{a}
5. Backfilling with Biodentine	15	814.54 ± 138.54^{c}

The same superscripted letters indicate no significant differences ($P > 0.05$).

Discussion

Immature teeth lack dentin wall thickness when compared to teeth that are fully developed. As the dentin wall thickness decreases, the resistance to fracture decreases and it becomes more dependent on the reinforcing capability of the materials used to restore the tooth[20]. Previous studies have evaluated the fracture resistance of immature teeth restored using various root canal filling methodologies, such as fiber post, gutta-percha, MTA, and composite resin[2,12,21]. In the present study, we aimed to test the reinforcing capability of Biodentine that can be used as an alternative to the use of MTA as apical plug material in apexification.

A significant concern regarding selection of a material to use for an apical plug is cytotoxicity of the material, because cytotoxicity may influence the viability of periradicular cells and cause cell death by apoptosis or necrosis[22,23]. Currently, there is limited information available about the cytotoxicity of Biodentine to periodontal cells. Recently, one study showed that Biodentine caused a gingival fibroblast reaction similar to that of MTA[24].

Maxillary central incisor teeth were selected for this study, as they are more susceptible to trauma and external impacts owing to their localizations. Teeth with similar dimensions at the CEJ were included in the experimental procedure to ensure standardization. Simulation of immature teeth formation was achieved using Peeso reamers, comparable to other studies[25,26]. However, this technique has potential shortcomings in that it may not fully replicate a real unformed root. It has been indicated that in pulpless immature teeth, as root dentinogenesis is halted, depending upon the stage of root development, the thin root wall has incompletely developed peritubular and intertubular dentin with higher tubular density toward the cementum. When mature teeth are enlarged to simulate immature teeth, the outer part of their roots would demonstrate lower tubular density and more intertubular dentin[27]. Therefore, this could mean that although these teeth may morphologically mimic immature teeth, they are unable to do so in terms of tissue composition or physical characteristics[13]. Some investigators applied the load at an angle of 45° to the long axis of the tooth to simulate the average angle of contact between maxillary and mandibular incisors in a class I occlusion[20,28–30]. In the present study, we also applied the compressive load with the same angle to test the fracture resistance of teeth with simulated immature apex.

Studies reported in literature have examined fracture resistance of teeth reinforced with fiber post, and the results have been mixed. Tanalp *et al.*[13] found that immature teeth reinforced with fiber posts were significantly stronger than unrestored teeth, but no stronger than those restored with gutta-percha. The results of another study showed that the fracture resistance values of teeth restored with fiber post were higher than that both the unprepared group and the group backfilling with gutta-percha[12]. Similarly, the results of a recent study showed that teeth restored with fiber posts had the higher fracture resistance than that restored with gutta-percha after apexification with MTA[31]. The findings of the present study were compared with the findings of studies conducted with MTA because there has been no study on fracture resistance of simulated immature teeth with an apical plug using Biodentine. In the current study, the fracture resistance values of teeth restored with fiber posts were higher than the groups that were unrestored, restored with gutta-percha, and restored using Biodentine. On the other hand, the values of the fiber post group were similar to those of the unprepared group (Table 1). This may be related to the similar modulus of elasticity for fiber posts and dentin. Actually, it was to be expected that Biodentine, which can be used as a dentin substitute according to Biodentine manufacturer's, had fracture resistance values similar to those of the unprepared group. One possibility for no difference between the fiber post group and the unprepared group may be because the fiber posts may more evenly distribute forces along the root[12].

In a recent study, Sawyer *et al.*[14] examined whether prolonged contact of dentin with 2 recently introduced calcium silicate-based materials, Biodentine and MTA Plus, adversely affects flexural properties. They stated that dentin flexural strength for dentin exposed to Biodentine decreased significantly after 2 and 3 months, whereas that exposed to MTA Plus decreased significantly after 3 months of aging. Also, they stated that the fracture resistance of roots will probably not adversely affect when these calcium silicate-based materials are used as apical plug material. However, the practice of completely obturating root canals with these new calcium silicate-based materials may decrease the fracture resistance of teeth. In the present study, storage time of specimens before the fracture testing was 2 weeks. The fracture resistance of teeth performed backfilling with Biodentine was similar to those of backfilling with gutta-percha. This may be attributed to short contact time (2 weeks) of dentin with Biodentine. Leiendecker *et al.*[32] evaluated that whether prolonged contact of mineralized dentin with Biodentine and MTA Plus adversely affects dentin collagen matrix integrity. The findings of their study showed that prolonged contact of mineralized dentin with Biodentine and MTA Plus has an adverse effect on the integrity of the dentin collagen matrix. Therefore, caution is advised when calcium silicate-based materials are used to obturate root canals with thin dentinal walls and in filling the full length of the canal to prevent collagen degradation that might lead to root fracture. A retrospective study conducted by Cvek[33] showed that endodontically treated immature teeth have a relatively high incidence of cervical root fracture. The *in vitro* studies evaluating the fracture resistance of immature teeth restored with various materials also confirmed this information. The findings of these *in vitro* studies showed that the fractures commonly occurred in the cervical third[2,12,21]. Regarding the fracture mode observed in the current study, fracture above the CEJ, at the CEJ, and mid-root fracture were the types of fractures detected. The fracture occurrence above the CEJ in the fiber post group can be an advantage for restoration without an additional treatment approach (i.e., surgical intervention) of a fractured tooth when compared with the other experimental groups which had fractures below CEJ.

Conclusion

Within the limitation of this *in vitro* study, it may be concluded that backfilling with gutta-percha, a fiber post, or Biodentine does increase the force required to fracture in simulated immature teeth performed with a Biodentine apical plug. However, this study indicates that fractured teeth backfilled with fiber post would be more easily restorable than teeth than the other groups. Further research is required to validate the findings obtained in this study. Moreover, the experimental studies comparing the fracture resistance of immature teeth restored with Biodentine or MTA may be helpful for making decisions concerning treatment plans for teeth with immature root formation.

Why this paper is important to pediatric dentists:

- There is no study concerning the fracture resistance of simulated immature teeth after the use of Biodentine as apical plug material.
- This paper provides that backfilling materials alter the fracture resistance of immature teeth after placement of Biodentine apical plug.

Conflict of interest

The authors declare no conflict of interest.

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