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Effect of different materials and canal filling methods in apexification of simulated immature teeth on fracture resistance–in vitro study

Salih Düzgün[1], İpek Eraslan Akyüz[1*] and Mehmet Çiçek[1]

Abstract

Background The restoration of immature teeth with thin dentinal walls remains a clinical challenge, and the choice of root canal filling material may influence their resistance to fracture. This in vitro study aimed to evaluate the fracture resistance and fracture patterns of simulated immature roots filled with different root canal filling materials and coronal backfilling strategies.

Methods Sixty-four simulated immature maxillary central incisors were standardized and randomly allocated into eight groups: negative and positive controls, TotalFill RRM Putty or Biodentine used alone, or each material combined with either thermoplasticized gutta-percha or a dual-cure composite resin (Grandio Core). Following apexification procedures, fracture resistance was measured using a universal testing machine, and fractures were classified as restorable or unrestorable. Statistical analyses included Welch ANOVA with Games–Howell post-hoc tests and Fisher's Exact Test for fracture type evaluation.

Results Significant differences in fracture resistance were observed among groups ($p < 0.001$). The positive control group demonstrated the lowest resistance. In contrast, the TotalFill RRM Putty + gutta-percha, Biodentine, and Biodentine + gutta-percha groups exhibited fracture resistance values comparable to those of the negative control ($p > 0.05$). Groups restored with gutta-percha showed higher fracture resistance than those restored with composite resin. Fracture mode distribution also differed significantly among groups ($p = 0.047$).

Conclusions Root canal filling materials and coronal backfilling techniques have a significant influence on the biomechanical behavior of immature teeth. Thermoplasticized gutta-percha demonstrated superior reinforcement compared with dual-cure composite resin, while Biodentine and TotalFill RRM Putty exhibited comparable mechanical performance. Material selection and obturation strategy play a crucial role in enhancing the structural integrity of immature roots during apexification procedures.

Keywords Apexification, Fracture resistance, Fracture type, Root canal filling material

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Introduction

Apical periodontitis in immature permanent teeth develops secondary to pulpal necrosis, typically following trauma or deep caries, and may lead to root development arrest and complications, including tooth loss, mandibular growth disturbances, impaired mastication, speech difficulties, and aesthetic problems. Preventing these outcomes requires appropriate clinical intervention. Apexification, a nonsurgical procedure that induces a calcific barrier at the open apex of non-vital teeth, helps block toxins and bacteria while facilitating root canal sealing [1]. However, the ideal material for apexification remains undetermined. These cases remain challenging due to open apices and thin dentinal walls, with earlier treatments including custom-fitted gutta-percha, paste fills, and apical surgeries [2].

In addition to apexification, regenerative endodontic procedures (REPs) such as revitalization have emerged as biologically advantageous alternatives that promote continued root development and increased dentin thickness. Nevertheless, apexification remains essential in cases where regeneration is not feasible or predictably successful [3].

Calcium silicate–based materials are widely used in apexification procedures due to their favorable biological properties and clinical applicability. Compared with calcium hydroxide, these materials form an apical barrier more quickly and allow single-visit treatment, thereby reducing the risk of complications associated with prolonged therapy. In addition, they exhibit improved sealing ability and bioactivity, which may contribute to more predictable treatment outcomes [4, 5]. However, despite these advantages, previous studies have reported comparable overall clinical success rates between calcium silicate–based materials and calcium hydroxide [6].

The ideal material and obturation strategy capable of providing both optimal apical healing and reinforcement of structurally compromised roots remains a matter of ongoing investigation. Previous studies have evaluated the efficacy of various materials in enhancing the fracture resistance of immature teeth, yielding mixed findings [7, 8]. Following apical plug placement using MTA, Biodentine, or gutta-percha, the mechanical performance of teeth can be further improved using reinforcement strategies such as bonded composite resins or fiber posts [9]. Several studies using various composite resins, including flowable formulations, have demonstrated promising improvements in fracture resistance [7, 8].

However, despite these contributions, current evidence remains limited regarding the biomechanical performance of recently introduced calcium silicate–based putties (Biodentine, TotalFill RRM Putty) when used in conjunction with contemporary reinforcement techniques. Most previous research has focused on MTA-based apexification, with insufficient attention to alternative putty formulations. Moreover, although composite resins have been investigated for root reinforcement, the specific effects of dual-cure composites, which can achieve chemical polymerization in regions inaccessible to light, remain largely unexplored. Dual-cure materials, such as Grandio Core, provide improved polymerization in deep, narrow canals, thereby minimizing bond strength discrepancies and enhancing homogeneity [10].

Recent literature has investigated several restorative strategies for immature teeth, with Crozet et al. [11] demonstrating that the fracture resistance of simulated immature maxillary anterior teeth varies depending on whether they are restored with different canal filling materials, micro-posts, or fiber posts. While this study provided valuable insights into reinforcement approaches for structurally compromised immature roots, it did not assess the biomechanical performance of newly developed calcium silicate–based putties. Furthermore, no previous investigation has directly compared these new-generation putty formulations, such as Biodentine and TotalFill RRM Putty, when combined with distinct coronal backfilling strategies (dual-cure composite versus thermoplasticized gutta-percha) within a standardized apexification model. This gap highlights the need for studies that evaluate both material selection and obturation technique in the context of immature root reinforcement.

Therefore, the present study aims to comprehensively evaluate the fracture resistance of immature teeth restored with Biodentine or TotalFill RRM Putty, either used alone or as an apical plug, followed by backfilling with dual-cure composite resin or thermoplasticized gutta-percha.

The first null hypothesis of this study is that different root canal filling materials would have no significant effect on the fracture resistance of immature permanent teeth. The second null hypothesis is that different root canal filling materials would have no significant impact on the types of fractures that occur.

Materials and methods

Sample size calculation

The sample size was calculated using Turcosa software (Turcosa Analytics Ltd. Co., Türkiye). The minimum number of specimens per group was determined based on the effect size and variability values reported in a previous study on fracture resistance in apexification models [12]. Power analysis (power = 0.90, effect size = 0.58, α = 0.05) indicated that each group required a minimum of eight specimens. The study was designed and reported in accordance with the PRILE 2021 guidelines for laboratory research in endodontology [13], and the experimental workflow is illustrated in Fig. 1.

Ethical approval was obtained from the institutional scientific review committee (Date: 30 April 2025, Approval No: 2025/210). All procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki. The extracted teeth were obtained

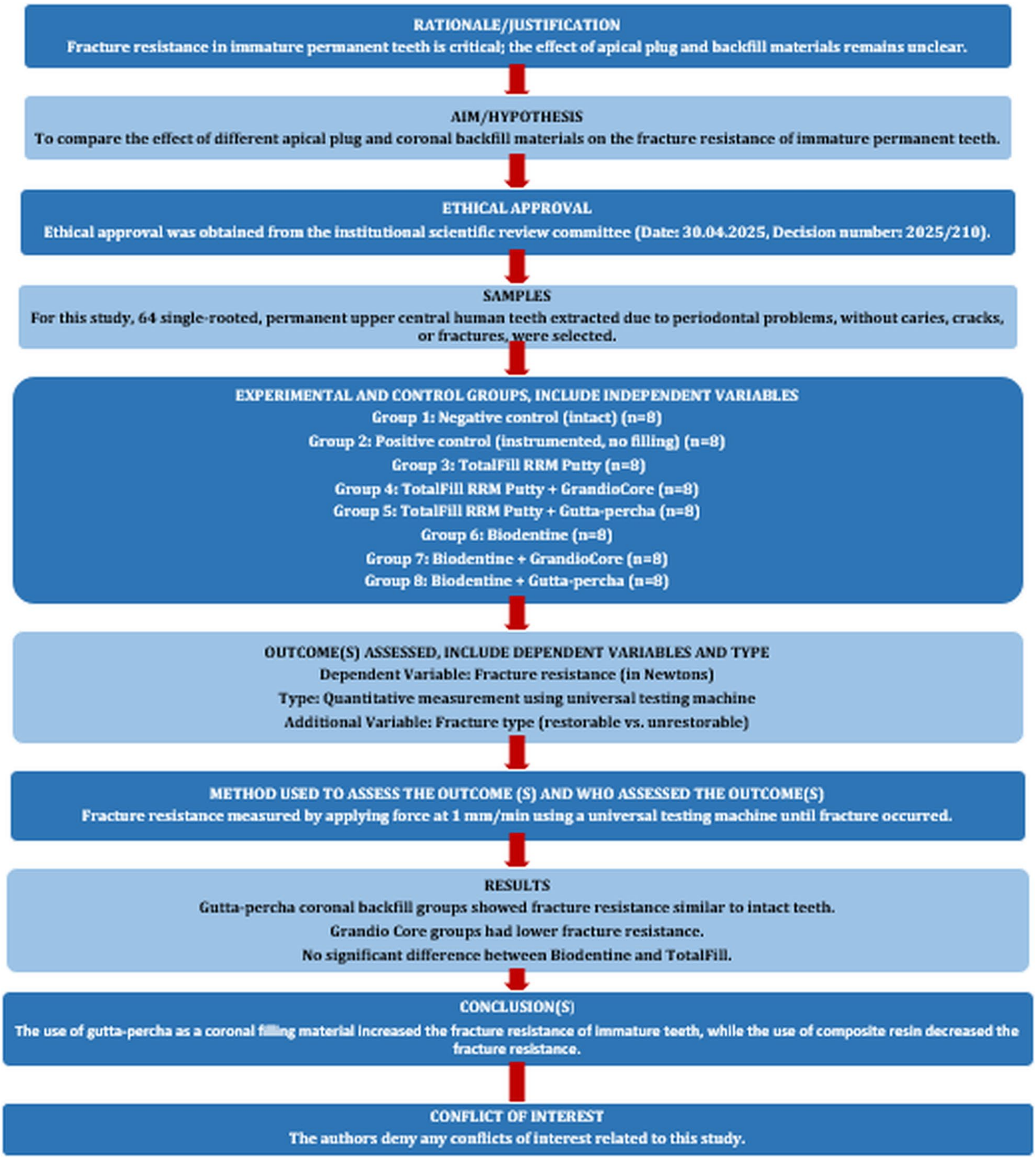

Fig. 1 Prile 2021 flowchart. *From: Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Roças IN, Siqueira JF Jr, Priya E, Jayaraman J, Pulikkotil SJ. Camilleri J, Boutsioukis C, Rossi.Fedele G, Dummer PMH (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontology: a consensus-based development. International Endodontic Journal May 3. https://onlinelibrary.wiley.com/doi/abs/101111/ici.13542. For further details visit: http://pride.endodonticguidelines.org/prile

with the patients' written informed consent at the time of extraction.

Sixty-four extracted maxillary central incisors without cracks, caries, or restorations were collected. To ensure homogeneity, buccolingual and mesiodistal root dimensions were measured 2 mm above the cementoenamel junction (CEJ) using a digital caliper (Mitutoyo, Hampshire, UK). Teeth showing more than 20% deviation from the mean were excluded. Standardized root lengths (13 ± 1 mm) were achieved by removing the crowns approximately 2 mm above the CEJ and reducing the apical portion according to previously established protocols [12].

Root canal preparation

Access cavities were prepared using a round bur and a high-speed handpiece. Working length was established by advancing a size 15 K-file (Dentsply Sirona) until visible at the apical foramen and then subtracting 1 mm. Canals were shaped with the ProTaper Next system (Dentsply Maillefer, Ballaigues, Switzerland) up to size X5 (#50).

To create an immature apex model, the canals were sequentially enlarged with Peeso reamers #1–6. The final reamer (#6) was extended 1 mm beyond the root end, following standard laboratory simulation techniques frequently used in apexification research.

Throughout instrumentation, irrigation was performed with 10 mL of 2.5% sodium hypochlorite using a 30-gauge side-vented needle. The final irrigation solution consisted of 5 mL of 17% EDTA and 10 mL of distilled water.

Ultrasonic activation of the irrigant was performed using the Ultra X device (Changzhou Sifary Medical Technology Co., Ltd., China) with a flexible X-Silvertip (#25/0.02) positioned 1 mm short of the working length. After drying, calcium hydroxide paste (UltraCal XS; Ultradent, USA) was placed into each canal to simulate clinical conditions. The access cavities were temporarily sealed (Cavit; 3 M ESPE, USA), and specimens were stored for seven days at 37 °C and 100% humidity.

After incubation, the temporary restorations were removed. Calcium hydroxide was flushed out using 10 mL of 5.25% sodium hypochlorite followed by 5 mL of 17% EDTA and a final rinse of 10 mL distilled water. Canals were dried with paper points.

Experimental groups

All procedures, including apical plug placement and subsequent coronal restoration, were performed under ×3.5 magnification using dental loupes.

To reduce the risk of contamination, all specimens were handled under controlled laboratory conditions. The canal walls were irrigated and dried with sterile paper points prior to material placement, and no temporary materials were used before the core build-up procedure.

Specimens were randomly assigned to eight groups ($n = 8$):

Negative control: No instrumentation or filling procedures were performed.
Positive control: Canals were prepared but left unfilled.
TP group (TotalFill RRM Putty): TotalFill RRM Putty (FKG Dentaire SA, Switzerland) was delivered into the canal and manually compacted to the level of the CEJ.
TP+GC group (TotalFill RRM Putty + Grandio Core): A 4-mm TotalFill RRM apical barrier (VOCO, Germany) was created and confirmed radiographically. After setting, a universal adhesive system (Futurabond U; VOCO, Germany) was applied to the canal walls according to the manufacturer's instructions prior to composite placement in the GC groups. Grandio Core was placed using the QuickMix system in 2–3 mm increments, each polymerized for 40 seconds using an LED curing unit (Valo Cordless; Ultradent, USA).
TP+GP group (TotalFill RRM Putty + gutta-percha backfill): A 4-mm TotalFill RRM apical plug was placed and verified radiographically. AH Plus sealer (Dentsply Sirona) was applied, and canals were backfilled with thermoplasticized gutta-percha (Eighteeth system, China) followed by vertical compaction.
BD group (Biodentine): Biodentine (Septodont, France) were triturated for 30 seconds and compacted into the canals up to the CEJ.
BD+GC group: Procedures matched those described for the TP+GC group, with TotalFill replaced by Biodentine.
BD+GP group: Procedures matched those described for the TP+GP group.

Standardized immature tooth models with open apices were used to simulate clinical conditions. Apical plug placement was performed using an incremental technique, in which the material was delivered in small portions and condensed stepwise using hand pluggers to ensure adequate adaptation and density. The adequacy of condensation and plug thickness was verified radiographically. All procedures were performed by a single experienced operator.

Periapical radiographs confirmed the quality of the fillings. Prior to coronal composite restoration, a universal adhesive system (Bond Force II; Tokuyama Dental, Tokyo, Japan) was applied to the intracoronal dentin surfaces in accordance with the manufacturer's instructions to ensure proper bonding. All teeth were restored with nanohybrid resin composite (Tokuyama Estelite Sigma

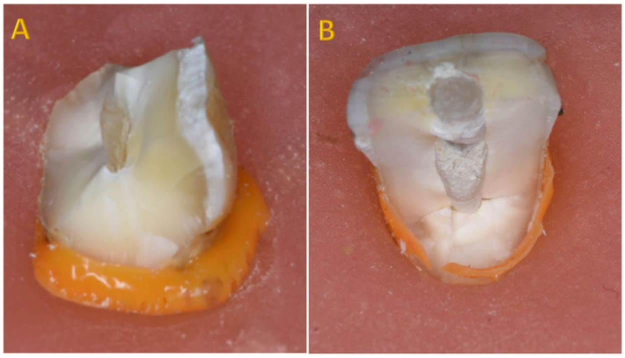

Fig. 2 The samples of restorable fracture in (**A**) group BD+GC and the unrestorable fracture in (**B**) group TP. BD, Biodentine; GC, Grandio Core; TP; TotalFill RRM Putty

Quick, Japan) and stored at 37 °C and 100% humidity for four weeks to ensure complete material maturation.

Fracture resistance testing

A uniform 0.2-mm layer of silicone impression material (Coltene/Whaledent AG, Altstätten, Switzerland) was applied to each root surface to simulate periodontal ligament elasticity. The roots were vertically embedded in acrylic resin blocks so that the CEJ remained 1.5–2 mm above the resin surface, mimicking alveolar bone level.

Fracture resistance was tested with a universal testing machine at a crosshead speed of 1 mm/min. The load was applied to the palatal surface above the cingulum at a 45° angle and centered mesiodistally, consistent with established testing protocols [14]. Fracture loads were recorded in Newtons (N).

Fracture patterns were categorized as:

Restorable: fracture line above the acrylic resin level
Unrestorable: fracture extending below the simulated bone level (Fig. 2).

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed for each group using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Results indicated that the assumption of normality was satisfied in all groups ($p > 0.05$). Descriptive statistics (mean and standard deviation) were calculated for continuous variables. Since the Levene test indicated that the variances were not homogeneous, the Welch ANOVA test was employed for group comparisons, and the Games–Howell post-hoc test was used to determine within-group differences.

The distribution of fracture types was assessed using the Pearson Chi-square test. Due to low expected cell counts (all below 5), additional analyses were performed with the Likelihood Ratio Chi-square test and Fisher's Exact Test to account for violations of test assumptions. Pairwise comparisons between groups were conducted using Fisher's Exact Test. Standardized residuals were examined to identify cells that contributed most to the significant differences. Statistical significance was set at $p < 0.05$.

Results

The descriptive statistics of fracture resistance values and the distribution of fracture types for all groups are presented in Table 1. Normality tests confirmed that the fracture resistance data were normally distributed across all groups ($p > 0.05$). Levene's test indicated a violation of the homogeneity of variances for fracture resistance ($p < 0.05$). Welch ANOVA results revealed significant differences in fracture resistance between the groups ($F(7, 23.745) = 8.110$, $p < 0.001$). The distribution of fracture resistance values across the experimental groups is illustrated in Fig. 3. According to the

Table 1 Fracture resistance values (in newtons), Standard Deviation (SD)

Groups	Fracture Resistance (*N*) Mean ± SD ($P = 0.000$)	Distribution of Fracture Types (n/%) ($P = 0.055$) Restorable Unrestorable
Negative Control	847.66 ± 210.72^{A}	5 / 7.8% 3 / 4.7%
Positive Control	266.36 ± 111.64^{D}	0 / 0% 8 / 12.5%
TotalFill	546.97 ± 140.75^{BC}	5 / 7.8% 3 / 4.7%
TotalFill + Grandio Core	392.72 ± 112.41^{C}	6 / 9.4% 2 / 3.1%
TotalFill + Gutta-percha	691.12 ± 239.69^{AB}	3 / 4.7% 5 / 7.8%
Biodentine	641.72 ± 108.30^{AB}	4 / 6.3% 4 / 6.3%
Biodentine + Grandio Core	444.53 ± 159.54^{C}	5 / 7.8% 3 / 4.7%
Biodentine + Gutta-percha	661.89 ± 326.50^{AB}	2 / 3.1% 6 / 9.4%
Total	561.62 ± 251.70	30 / 46.9% 34 / 53.1%
Statistical tests	**Welch ANOVA**	**Chi-square**

* Different superscript capital letters indicate statistically significant differences between groups based on Welch ANOVA followed by Games–Howell post hoc test ($p < 0.05$). Groups sharing the same letter are not significantly different

* *ANOVA* analysis of variance, *N* Newton, *SD* Standard Deviation, *n* Number

Values sharing the same superscript letter are not significantly different, whereas values with different letters indicate statistically significant differences between groups (Welch ANOVA followed by Games–Howell post hoc test, $p < 0.05$)

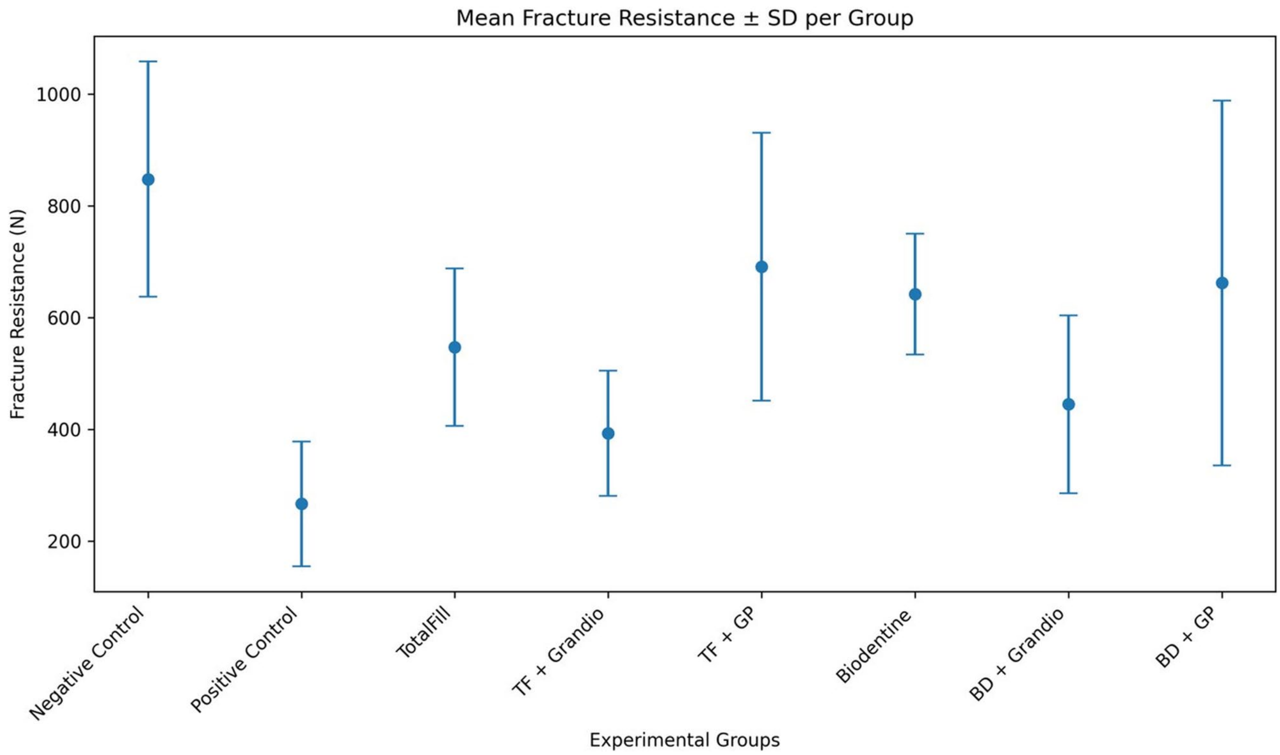

Fig. 3 Distribution of fracture resistance values (mean + standard deviation) across the experimental groups

Games-Howell test results, the negative control group showed significantly higher fracture resistance than the positive control ($p = 0.001$), TP + GC ($p = 0.004$), and BD + GC ($p = 0.014$) groups. The positive control had the lowest fracture resistance of all groups. Groups in which root canal fillings were combined with gutta-percha exhibited higher fracture resistance compared to those combined with Grandio Core ($p < 0.05$).

When examining the distribution of fracture types across groups, the Pearson Chi-square test did not show a statistically significant difference ($\chi^2(7) = 13.804$; $p = 0.055$). However, because the expected counts in all cells were below the recommended minimum, the Likelihood Ratio test was applied and revealed a significant difference instead ($\chi^2(7) = 17.05$; $p = 0.017$). Fisher's Exact Test further confirmed this finding ($p = 0.047$). These results suggest that the pattern of restorable versus unrestorable fractures varies meaningfully between the experimental groups.

Upon closer examination, the positive control group had fewer restorable fractures than expected (standardized residual = −1.9). Pairwise comparisons using Fisher's Exact Test indicated that this group differed significantly from the TP + GC ($p = 0.0069$) and BD + GC ($p = 0.0256$) groups. No other significant differences were observed among the remaining groups ($p > 0.05$).

Discussion

Cold lateral condensation is historically considered the benchmark obturation technique; however, its inability to create a homogeneous mass and its tendency to leave voids and gaps between cones have been widely criticized [15]. This inherently compromises the apical seal and may predispose teeth to structural weakness. Warm obturation techniques were developed to address these limitations and have shown improved homogeneity and adaptation, although their effect on fracture resistance remains inconsistent in the literature [16].

In some studies, composite resin materials have been preferred because of their color stability, dentin-like modulus of elasticity, and ease of application [9, 17]. In the present study, dual-cure composite resin (Grandio Core) and thermoplasticized gutta-percha were compared following apexification with Biodentine or TotalFill RRM Putty. This design enabled evaluation of not only the apical plug material but also the coronal filling strategy on the fracture resistance of weakened immature roots. The first null hypothesis was rejected, as the restorative approach significantly influenced fracture resistance.

The apexification model employed in this study, which involves apical enlargement using Peeso reamers, is widely validated and closely replicates the clinical anatomy of immature teeth, in accordance with previous studies evaluating fracture resistance after MTA or Biodentine application [18, 19]. The use of periodontal ligament simulation with silicone also increases biomechanical realism, as verified in earlier in vitro research [20].

Consistent with previous studies, the positive control group demonstrated the lowest fracture resistance, confirming that root canal preparation without subsequent reinforcement significantly weakens dentin structure. Çelikten et al. [21] and Punjabi et al. [22] similarly reported that unrestored immature teeth exhibit markedly reduced resistance to fracture compared with restored or intact teeth.

Although finite element analyses such as Chun et al. [23] suggest that composite backfilling increases stress concentration in cervical regions, such models remain limited by assumptions about material behavior, periodontal support, and loading conditions. In clinical reality, polymerization shrinkage, disruption of the dentin–adhesive interface, and microvoid formation may explain the lower fracture resistance observed in composite backfilling groups in the present study.

The TP + GP, BD + GP, BD-only, and TP-only groups demonstrated comparable fracture resistance in the present study. The existing literature on calcium silicate–based materials in immature teeth reports heterogeneous findings, largely influenced by differences in material properties, experimental design, and reinforcement strategies [18, 19].

Interestingly, gutta-percha backfilling resulted in fracture resistance values closest to those of intact teeth. Although gutta-percha does not chemically bond to dentin and exhibits a relatively low elastic modulus [24], its clinical performance may vary depending on the obturation technique. Warm obturation techniques have been suggested to improve the homogeneity and adaptation of filling materials; however, their influence on fracture resistance remains inconclusive in the literature [16, 24].

While previous studies have sometimes suggested that dual-cure composites yield higher fracture resistance than gutta-percha [25, 26], the results of the present study show the opposite trend. Such discrepancies may arise from differences in canal geometry, polymerization depth, adhesive protocols, and loading methods. In deep, narrow canals, the polymerization of composite materials may be affected by limited light transmission.

The significantly lower proportion of restorable fractures in the positive control group further indicates the crucial role of root canal filling materials in modifying fracture behavior. Previous studies have demonstrated that different restorative strategies may influence not only fracture resistance but also the mode of failure, with certain approaches leading to more favorable (restorable) fracture patterns while others result in catastrophic failures [9, 27]. Thus, the second null hypothesis was rejected.

From a clinical standpoint, not only fracture resistance but also the mode of failure should be considered when evaluating restorative strategies for immature teeth. In the present study, although the groups restored with Grandio Core in combination with apical plug materials exhibited relatively lower fracture resistance values, they tended to demonstrate more favorable fracture patterns in terms of reparability. This may represent a clinically relevant advantage, as restorable fractures allow preservation of tooth structure and facilitate retreatment [9].

In contrast, groups backfilled with thermoplasticized gutta-percha generally showed higher fracture resistance; however, the fracture patterns observed in these groups were less favorable, often resulting in non-restorable failures. This finding suggests that greater fracture resistance does not necessarily correspond to improved clinical prognosis. Therefore, both mechanical strength and failure mode should be considered when selecting restorative approaches for structurally compromised immature teeth [11].

One limitation of the present study is that aging procedures, such as thermocycling or cyclic fatigue loading, were not performed. As a result, the findings mainly reflect the immediate mechanical behavior of the tested materials rather than their long-term clinical performance. Future studies incorporating aging protocols and larger sample sizes may provide more clinically relevant insights by better simulating intraoral conditions.

In addition, further investigations into different reinforcement approaches, including fiber-post systems, could contribute to a more comprehensive understanding of how structurally weakened immature teeth can be effectively reinforced following apexification procedures.

Overall, the present study provides novel biomechanical insights into an area where current evidence remains limited. By evaluating contemporary calcium silicate–based putties in combination with two distinct coronal backfilling strategies within a standardized apexification model, this study addresses a notable gap in the existing literature.

Conclusions

Within the limitations of this in vitro study, gutta-percha as a coronal backfilling material yielded fracture resistance values close to those observed in intact teeth, whereas dual-cure composite resin showed lower resistance. Biodentine and TotalFill RRM Putty yielded similar fracture resistance outcomes. Nevertheless, these results should be interpreted cautiously, as differences in material characteristics may affect their clinical use.

Abbreviations

GC	Grandio Core
MTA	Mineral trioxide aggregate
N	Newton
REPs	Regenerative endodontic procedures
CEJ	Cementoenamel junction
TP	TotalFill RRM Putty
GP	Gutta-percha
BD	Biodentine
CBCT	Cone-Beam Computed Tomography

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Relevant reporting guidelines
Paperwork
PRILE 2021 guidelines.

Authors' contributions
Salih Düzgün: Supervision, Project administration, Resources, Funding acquisition. İpek Eraslan Akyüz: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. Mehmet Çiçek: Data curation, Formal analysis, Software, Visualization.

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Data availability
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval was received for the current study from the ethics committee of Erciyes University Faculty of Medicine (Decision number: 2025/210). The study protocol was designed in accordance with the principles of the Declaration of Helsinki. The treatment process, side effects, and alternative treatment options were explained to the patients, and informed consent was obtained through their signatures.

Consent for publication
Not applicable. This study did not involve human participants or individual personal data requiring consent for publication.

Competing interests
The authors declare no competing interests.

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