Original Research

Comparative Evaluation of the Vertical and Horizontal Root Fracture Resistance of Primary Maxillary Incisors Obturated with Zinc Oxide Eugenol, Vitapex, and Endoflas: An *In Vitro* Study

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Abstract

Aim: To evaluate and compare the vertical and horizontal root fracture resistance of primary maxillary incisors obturated with zinc oxide eugenol, vitapex, and endoflas. **Materials and Methods:** The present research is an *in vitro* comparative study. Eighty extracted primary maxillary incisors, selected by random sampling method were allocated to four groups depending on the type of material used for obturation, Group I: Control group. Group II: Zinc Oxide Eugenol. Group III: Vitapex Group IV: Endoflas. Specimens were loaded vertically and horizontally by a conical spreader tip affixed to an Instron testing machine until the root fractured. The load at fracture and the pattern of fracture were recorded. **Results:** Results were compiled and analyzed statistically by using one way ANOVA followed by Tukey's *post hoc* Test and Unpaired t-test for comparison of force between amongst groups. The load at which fracture occurred was significantly higher for Endoflas followed by Zinc oxide eugenol, Vitapex, and was least in the control group. **Conclusion:** Within the limitations of this in-vitro research, it can be concluded that both the vertical and horizontal root fracture resistance was highest in Endoflas followed by Vitapex and zinc oxide eugenol which showed similar fracture resistance and was least in the control group. The proposed obturating materials provided resistance to occlusal load that is higher than the physiologic chewing force in children.

Keywords: Calcium Hydroxide, Endoflas, Pulpectomy, Root Canal Filling Materials, Zinc Oxide Eugenol Cement

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Introduction

Pulpectomy is a conventional treatment method to prevent the premature loss of primary teeth severely destroyed by trauma or ECC.[1] It helps to salvage damaged teeth but there are certain disadvantages of this endodontic treatment as the teeth become weak and susceptible to fracture. Secondly decrease in water content of teeth occurs due to exposed dentinal tubules, making it brittle as there is decrease in dentin elasticity.[2] Thus, to overcome these unfavorable treatment outcomes, the teeth are obturated with a biocompatible material that aids in functional restoration of the involved tooth. It is imperative in case of primary anterior dentition that the obturating material helps to strengthen the tooth structure to a certain extent as the incidence of falls is much more in primary dentition than the permanent dentition due to

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underdeveloped motor skills thus causing trauma to the anterior primary dentition.[2]

The success of pulpectomy in primary teeth relies on selecting the ideal root canal filling material. It is indeed tricky to choose the suitable appropriate filling materials for primary teeth as no one material fulfils all the criteria. Presently, the commonly used materials for primary root canal fillings are Zinc Oxide Eugenol, Calcium Hydroxide, Iodoform based pastes like Vitapex, Maisto's and Endoflas.

Zinc oxide-eugenol cement (ZOE) has long been used as a root canal filling material for deciduous teeth, and in a survey conducted in 1997 it was quoted as the desired root canal filling material by 94% of the chairs of predoctoral pediatric dental programs in the Unites States.[3] Endoflas is a resorbable paste comprising elements similar to that of vitapex, with an add-on of zinc oxide eugenol. Fuks *et al.* noted that Endoflas resorbed when it extends periapically, however, it did not resorb intra radicularly and reported 70% success clinically with endoflas and a 100% reduction in periapical radiolucency. The drawback of the material is its eugenol content that can cause periapical irritation and can lead to tooth discoloration.[4] Vitapex is a syringe-loaded viscous pre-mixed paste comprising Iodoform and Calcium hydroxide.[4] Benefits of vitapex are that it has a convenient delivery system; It is radio-opaque and does not set to a hard mass. It has bone regeneration ability and diminishes atypical tooth mobility and pre-existent bone radiolucency. The success rates of Vitapex and ZOE were 100% and 78.5%.[5] As per a recently conducted meta-analysis it has been understood that due to its resorbable property, Ca(OH)2/iodoform is the best filling material to be utilized for pulpectomy in primary teeth nearing exfoliation. On the contrary, either ZOE or ZOE/iodoform combined with Ca (OH)2 are the materials of choice for pulpectomy in primary teeth that need long time before shedding.[6]

Many studies have assessed the fracture resistance of anterior primary teeth based on the properties of an optimal length of intracanal post irrespective of the obturating material being used.[7] Therefore, validating the need of the study as very little has been published concerning strength of primary dentition post treatment, we aim to compare the effect of obturating materials like ZOE, Vitapex and Endoflas on the fracture resistance of primary anterior teeth. The null hypothesis of the study will be $\mathbf{H_0}$: μ = There will not be any disparity in fracture resistance with various obturating materials.

It would be best if rapid, appropriate treatment using a suitable obturating material can reduce the impact of traumatic injury from both an oral health and an aesthetic standpoint by managing pain and preventing possible damage to developing permanent teeth.

Materials and Methods

Setting and design

The current study was an in-vitro comparative study conducted in Department of Pedodontics and Preventive Dentistry, People`s College of Dental Science and Research Centre and Praj Metallurgical Laboratory, Pune for the evaluation of fracture resistance using Instron Universal Testing Machine. The overall period for the study was 3 years.

Ethical approval and Informed consents

Study was conducted after obtaining ethical clearance from the institutional review board of People`s University (Ref/PCDS/Acad./12–13/3659 (6). The study has been accomplished in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standard. Study details were explained, and informed consent was obtained from all the patient's parents. The consent form was according to WHO Informed Parental Consent form for Research.

Sampling criteria

Study design consisted of 80 extracted human primary anterior teeth selected by random sampling process. The sample size was calculated based on the criteria of 85% power of calculation and a level of 0.05 for 2-sided test. This sample size was appropriate for the study that is suitable for obtaining statistically valid data. Over-retained single rooted primary anterior teeth with at-least 2/3rd root length were extracted and stored in distilled water which formed the inclusion criteria. The patient information was not gathered for confidentiality. Primary teeth with internal or external resorption, root with multiple canals, cracks and caries were excluded from the study after careful evaluation using a fiber optic light under stereomicroscope.

Randomization/Bias

Extracted teeth were kept in distilled water for not more than one month prior to the study. Teeth were kept hydrated all the time before the evaluation to avoid any bias in the results.

Methodology

The methodology was divided into following steps:

- Preparation of Samples
- Root Canal Preparation and Sterilization
- Root canal Obturation
- Assessment and Evaluation of Vertical and Horizontal root fracture resistance

Sample preparation

The crown portion was sectioned at the CEJ using a double-sided diamond disc under proper irrigation with

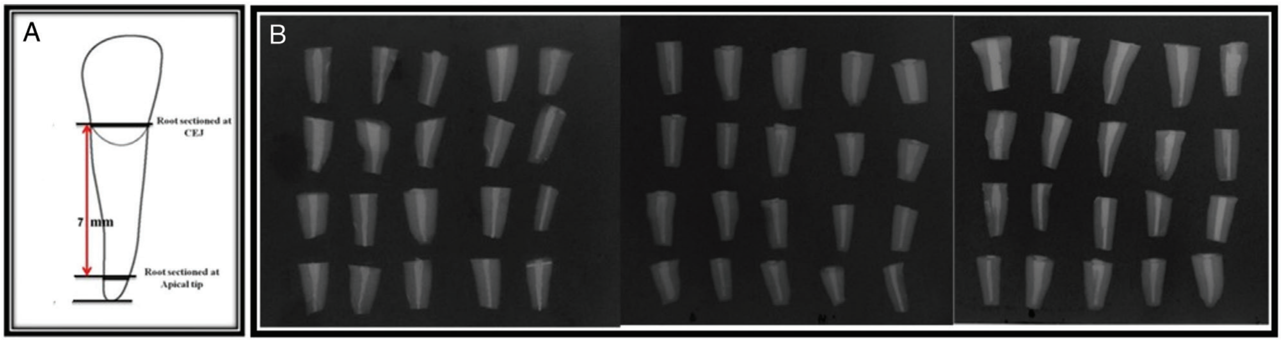

Figure 1: a: Final specimen sectioning. b: Radiographs confirming adequacy of sectioned roots

Table 1: Sample size grouping and allocation

Experimental Groups	Vertical Root Fracture Resistance	Horizontal Root Fracture Resistance
Group A: Control group ($n = 20$)	Subgroup I ($n = 10$)	Subgroup I ($n = 10$)
Group B: Zinc Oxide Eugenol (Prime dental products Pvt Ltd) ($n = 20$)	Subgroup II ($n = 10$)	Subgroup II ($n = 10$)
Group C: Vitapex (Neo Dental International) ($n = 20$)	Subgroup II ($n = 10$)	Subgroup III ($n = 10$)
Group D: Endoflas (Sanlor Laborataries) ($n = 20$)	Subgroup IV ($n = 10$)	Subgroup IV ($n = 10$)

distilled water. The root tips of all the specimens were further sectioned in such a way that the final specimens derived would measure 7 mm from the CEJ (cervical end) to the apical end. Measurement was done with help of digital vernier calliper. [Figure 1a]

Study methods

Root canal preparation and sterilization

Canals were prepared using an ISO #15 H file in a pull-back action and enlarged up to ISO #50 H file. After each instrument size, copious irrigation using a 25-gauge needle was done with 10 ml 5.25% sodium hypochlorite. The final irrigation was performed using 10 ml of normal saline. The canals were then dried using ISO #50 paper points. The samples were equally divided as shown in [Table 1].

Root canal obturation

For Group I (Control Group): No obturating material was used and access cavity was sealed with cavit. For Group II and Group III Using an ISO # 40 lentulo spiral mounted in a slow-speed hand piece, Zinc oxide eugenoland Endoflas were placed respectively. A rubber stopper was used to keep the lentulo spiral 1 mm short of the working length (7 mm). The process was repeated 5 to 7 times for each tooth until the canal orifice appeared filled. Access cavity was sealed with cavit. In Group IV Vitapex through a pre-filled syringe was placed and access cavity was sealed with cavit.

Radiographs of the roots were taken in both labiolingual and mesiodistal directions to confirm the adequacy of the root filling in terms of appropriate length, density, and taper for all the samples. [Figure 1b]

Samples were then wrapped in wet gauze to maintain a humid environment and stored for 14 days in individual coded glasses at 37˚C in an incubator to simulate in-vivo conditions.

Observational parameters

Observer's recruitment

The observers recruited was blinded while evaluating the fracture load

Analysis method and parameters needed to achieve

After 14 days the samples were removed from the incubator and entire root was covered with 200µm thick silicone paste layer up to 2 mm short of CEJ to simulate periodontal ligament. Each root was then set up vertically into an acrylic resin block (2 × 2x3cm) [Figure 2a] with the help of a metallic mould. Care was taken while placing the sample in resin to retain 2 mm of cervical portion above the resin and thus the investigator was blinded as well while evaluating the fracture load. A plunger was attached in the Universal Testing Machine and the sample was centered beneath the plunger on the lower plate [Figure 2b]. Plunger was propelled downward at a crosshead speed of 5 mm/min[8] parallel to the long axis of the tooth in the centre of the orifice until the root fractured. When the load suddenly decreased more than 25%, it was an indication that fracture had occurred. The fractured root was later examined under a stereo microscope with 10X magnification to ascertain the fracture pattern, which was categorized into bucco-lingual, mesiodistal and compound fracture. For horizontal root fracture shearing force in a lingual-labial direction was applied at the level of the lingual CEJ in an oblique direction. [Figure 2c]. The force was applied at a crosshead speed of 5 mm/minute at an angle of 130–150^0 to the long axis of the tooth.

Statistical analysis

This research was undertaken to assess vertical and horizontal root fracture resistance of primary maxillary anteriors.

Frequency (number), percentage, mean, standard deviation, minimum and maximum values of variables were calculated. Shapiro-Wilk test showed that forces values follow normal distribution hence parametric test One way ANOVA (analysis of variance) followed by Tukey post Hoc test and Unpaired t-test were used for comparison of force amongst separate groups. Nominal data (fracture pattern) was compared using Pearson's Chi-square test. P value <0.05 was considered statistically significant. Data analysis was done using Statistical Package for Social Sciences (SPSS) v.21 for windows.

Results

Vertical force (N) for root fracture in different experimental groups [Table 2]

One way ANOVA showed significant variation for vertical force (N) for root fracture in different experimental groups. Tukey post hoc test was applied for pairwise comparison, which showed that the vertical force needed for root fracture is significantly greater in Zinc oxide eugenol (min-203.87 and max-335.52 with a Mean±SD of 276.77 ± 39.68) and vitapex(min 207.60 and max 380.41 with a Mean±SD of 299.01 ± 56.28)when compared with control (min 98.87 and max 149.70 with a Mean±SD of 299.01 ± 56.28).Endoflas showed the highest force value (min-276.10 and max- 400.07 with a Mean±SD of 352.71 ± 41.33 when compared to control, vitapex and Zinc oxide eugenol. There was no significant difference between ZOE and Vitapex.

Horizontal force (N) for root fracture in different experimental groups [Table 3]

One-way ANOVA showed significant difference for horizontal force (N) for root fracture in different experimental groups. Tukey post hoc test was applied for pairwise comparison, which showed that force is significantly greater in ZOE(min-145.92 and max-492.25 with a mean ±SD of 307.56 ± 119.16) and Vitapex (Min-207.60 and Max-393.71 with a Mean ±SD of 287.05 ± 67.64) compared to Control (Min-91.29 and Max-214.71 with a Mean ± SD of 138.88 ± 43.39)Force

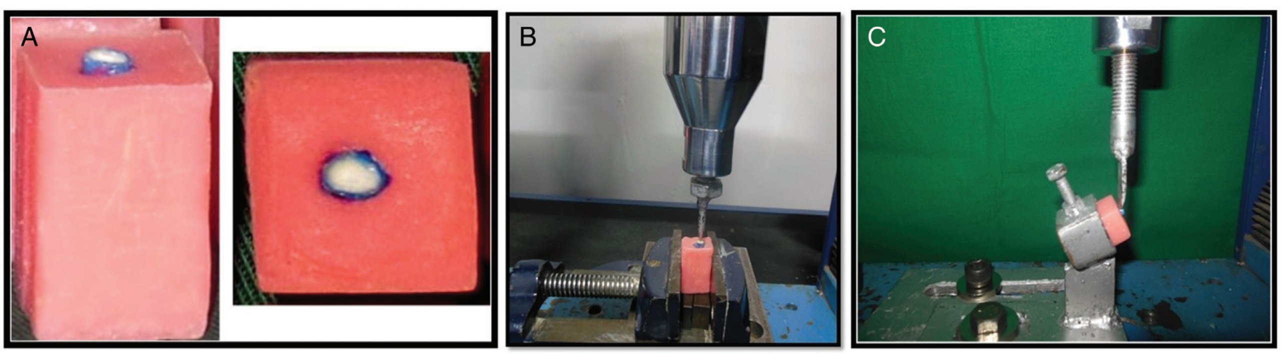

Figure 2: a: Specimens mounted in acrylic resin. b, c: Specimens mounted in the universal testing machine

Table 2: Mean, standard deviation, minimum and maximum values of vertical force (N) for root fracture in different experimental groups

Groups	Force (Newton)		One way ANOVA test	Tukey Post Hoc test (Significant results)
	Mean ± SD	Min-Max		
Control (n = 10)	120.32 ± 20.73	92.87–149.70	57.933 P = 0.000 (<0.05) Significant Difference	**ZOEandVitapex**> Control; **Endoflas**> ZOEand Vitapex>Control
ZOE (n = 10)	276.77 ± 39.68	203.87–335.52		
Vitapex(n = 10)	299.01 ± 56.28	207.60–380.41		
Endoflas(n = 10)	352.71 ± 41.33	276.10–400.07		

p <0.05-Significant.

is significantly greater in Endoflas (Min-304.07 and Max-502.93 with a Mean ± SD of 406.36 ± 75.26) than ZOE, Vitapex and Control. There was no significant difference between ZOE and Vitapex

Comparison of vertical and horizontal force (N) for root fracture in different experimental groups [Table 4]

Unpaired t-test showed that there was no statistically significant difference for vertical and horizontal force for root fracture among any experimental group. The Mean ± SD for vertical force of the control group was 120.32 ± 20.73 and for horizontal force was 138.88 ± 43.39. For Zinc Oxide Eugenol the Mean ± SD for vertical force was 276.77 ± 39.68 and horizontal force was 307.56 ± 119.16. For, vertical force of vitapex it was 299.01 ± 56.28 and for horizontal force 287.05 ± 67.64. The Mean± SD for vertical force of Endoflas was 352.71 ± 41.33 and for horizontal force it was 406.36 ± 75.26

Comparison of frequency of root fracture pattern due to vertical force (N) in different experimental groups [Table 5]

Root fracture pattern was categorized as multiple fracture lines [Figure 3a] and single fracture line which could be in mesio-distal or bucco-lingual direction [Figure 3b]. Chi-square showed that there was no significant difference for root fracture pattern due to vertical force among different experimental groups. For the control group single fracture

Table 3: Mean, standard deviation, minimum and maximum values of horizontal force (N) for root fracture in different experimental groups

Groups	Force (Newton)		One way ANOVA test	Tukey Post Hoc test (Significant results)
	Mean ± SD	Min-Max		
Control (*n* = 10)	138.88 ± 43.39	91.29–214.71	18.536 *P* = 0.000 (<0.05) Significant Difference	**ZOE andVitapex**> Control; **Endoflas**>ZOEandVitapex> Control
ZOE (*n* = 10)	307.56 ± 119.16	145.92–492.25		
Vitapex(*n* = 10)	287.05 ± 67.64	207.60–393.71		
Endoflas(*n* = 10)	406.36 ± 75.26	304.07–502.93		

P < 0.05-Significant

Table 4: Comparison of vertical and horizontal force (N) for root fracture in different experimental groups

Groups	Force (Mean ± SD, Newton)		Unpaired t-test	p value
	Vertical	Horizontal		
Control	120.32 ± 20.73	138.88 ± 43.39	-1.221	0.244 (>0.05), Not Sig.
ZOE	276.77 ± 39.68	307.56 ± 119.16	-0.775	0.455 (>0.05), Not Sig.
Vitapex	299.01 ± 56.28	287.05 ± 67.64	0.430	0.672 (>0.05), Not Sig.
Endoflas	352.71 ± 41.33	406.36 ± 75.26	-1.976	0.068 (>0.05), Not Sig.

p <0.05-Significant

Table 5: Comparison of root fracture pattern due to vertical and horizontal force (N) in different experimental groups

Groups	Force	Fracture Pattern					Chi-square test and p Value
		Single Fracture Line (Mesio-Distal)	Single Fracture Line (Bucco-Lingual)	Multiple Fractures	Avulsion	Total	
Control	Vertical n (%)	03 (30.0)	00 (0.0)	07 (70.0)	00 (0.0)	10 (100.0)	df= 2.067 p= 0.356 (>0.05), Not Sig.
	Horizontal n (%)	01 (10.0)	00 (0.0)	08 (80.0)	01 (10.0)	10 (100.0)	
ZOE	Vertical n (%)	02 (20.0)	01 (10.0)	07 (70.0)	00 (0.0)	10 (100.0)	3.077 df=3 p= 0.380 (>0.05), Not Sig.
	Horizontal n (%)	02 (20.0)	00 (0.0)	06 (60.0)	02 (20.0)	10 (100.0)	
Vitapex	Vertical n (%)	02 (20.0)	00 (0.0)	08 (80.0)	00 (0.0)	10 (100.0)	4.000 df=3 p= 0.261 (>0.05), Not Sig.
	Horizontal n (%)	00 (0.0)	01 (10.0)	08 (80.0)	01 (10.0)	10 (100.0)	
Endoflas	Vertical n (%)	02 (20.0)	00 (0.0)	08 (80.0)	00 (0.0)	10 (100.0)	2.892 df=2 p=0.235 (>0.05), Not Sig.

p <0.05-Significant

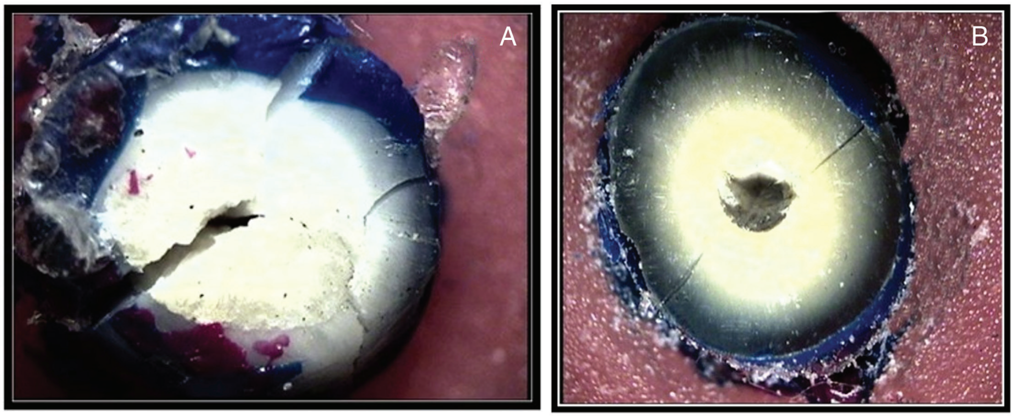

Figure 3: a: Specimen showing multiple fracture lines on stereomicroscopic evaluation. b: Specimen showing single fracture line on stereomicroscopic evaluation

line in a mesio-distal direction was seen in 3specimens and multiple fractures in 7 specimens. For zinc oxide Eugenol 3 specimens were seen with a single fracture line 2 in mesio-distal direction and 1 in bucco-lingual direction. Multiple fractures were seen in 7 specimens. In vitapex and endoflas out of the 10 specimens on which vertical force was applied, 2 specimens showed single fracture line in a mesio-distal direction and 8 specimens showed multiple fractures.

Discussion

Apart from the positive properties of the obturating materials that are harnessed for the success of the pulpectomy procedure the fact that goes unnoticed especially in case of primary dentition is the strength of the remaining tooth structure which is very important to retain the tooth in the arch after the pulpectomy procedure against the traumatic and the masticatory forces.[1] Hence this research was undertaken to assess the fracture resistance of primary teeth post treatment to determine the strength imparted to them by the obturating materials.

The samples utilized in this study were limited to over-retained primary maxillary central incisors obtained from patients. Although the difference in the root morphology and root dentine thickness may affect the load at root fracture, the strength of experimental teeth might be comparable because they were selected and stored in the same condition. In the present study, teeth were carefully selected for standardized size and absence of any root caries and cracks that may influence the resistance to fracture by acting as stress concentration areas.[9] The above properties of all samples were taken into consideration to ensure the same size and shape. Standardized preparations were used to produce dentinal walls of similar thickness by biomechanical preparation. All canals were enlarged up to ISO # 50 to ensure similar amount of obturating material in all the specimens. When mounting the teeth in the acrylic resin, attempts were made to mimic the periodontal ligament (PDL) by using a silicone paste. They represent comparable behavior when submitted to external stress, i.e., the response is nonlinear and viscous. After setting, the material is quite like the elastic modulus of human PDL. An attempt was made to standardize the thickness by applying a uniform layer of silicone paste (200µm).[10]

For vertical root fracture resistance, load was applied at an angle parallel to the long axis of the tooth and for horizontal root fracture resistance load was applied at an angle 130–150^0 to the long axis of the tooth to simulate the typical angle of contact between maxillary and mandibular incisors in a class I occlusion. The aim of the present study was not just to simulate traumatic fractures but also the masticatory load, so the concern was more towards the cervical root strength. Hence the applied force was flat to the lingual surface and was directed in a lingual–labial direction. The load was applied at a slow crosshead speed (5 mm/min).[8] The model used in our study proved successful, in that all fractures passed through the root apical to the CEJ and thus tested the root canal obturating material's ability to add strength to the prepared canals.

The values of all the experimental groups were higher than the control group. Thus, it can be concluded that all these materials play a very important role in substituting the lost part of the tooth and act as a reinforcement to increase the fracture resistance. Zinc Oxide Eugenol has compressive strength of 48mpa and tensile strength 4.1mpa.[6] In the present study the vertical and horizontal fracture resistance was highest for endoflas followed by ZOE and vitapex. The fracture resistance in the experimental groups was statistically higher than the control group. Thus, it can be concluded that ZOE to some extent provided reinforcement to the tooth structure on account of its mechanical properties. Vitapex is a combination of calcium hydroxide and iodoform. The results of load for vitapex at which fracture occurred were like the zinc oxide eugenol group. This could be attributed to the compressive strength of calcium hydroxide cement which is reported to be 10-27mpa after 24 hours which increases with time.[6] The incubation period of 14 days might have facilitated further increase in its strength and thereby the re-enforcement. The presence of oily vehicle also promotes lowest solubility and diffusion of calcium hydroxide pastes.[11] Root canal filling materials like pastes containing an oily vehicle, particularly those with an antibacterial substance (i.e., iodoform) have shown more favorable results than more soluble pastes, in primary teeth.[12]

The fracture load borne by Endoflas was significantly higher than ZOE and Vitapex at both vertical and horizontal loading which could be due to the combined mechanical properties of its constituents mainly ZOE and calcium hydroxide. Endoflas also firmly adheres to the surface of the root canals to provide a good seal.[13] It is suggested that materials that can adhere to the root canal dentin surface would strengthen the remaining tooth structure.[13]

Similar studies evaluating fracture resistance in primary teeth by using 3 different post types concluded that prefabricated glass fiber posts were far more fracture resistant, and they can be promisingly used for the restoration of anterior primary teeth.[14] Another study on fracture resistance of primary anterior teeth compared Bulk-Fill and a Conventional Composite and the combination of both for coronal restoration of severely damaged primary anterior teeth. They concluded that Bulk-fill composites can be used for coronal reconstruction of severely damaged primary anterior teeth like conventional composites to decrease the treatment time in pediatric patients[15]

Another accidental finding which was reported in the study was avulsion of some of the specimens without any fracture lines which could be attributed to the cushioning and shock absorbing property of the artificial substitute of the periodontal ligament simulating *in vivo* conditions. It has been reported that the minimum and maximum bite force in three- to six-year-olds at the posterior region were 5.00 newtons (N) and 353.64 N respectively.[16] The values obtained in the present study were 120.32 ± 20.73 N (Control group) and 352.71 ± 41.33 N (Endoflas) on vertical load. Both the values are for the bite force in the anterior region that are comparable to the values that have been reported for the bite force in the posterior region which shows that the obturating materials reinforce primary anterior teeth by enhancing the strength in some manner. Further *in vivo* studies should be undertaken to confirm their property. The minimum and maximum values for horizontal load were 138.88 ± 43.39 for the control group and 406.36 ± 75.26 for Endoflas. The values for horizontal load were also significantly higher than the bite force in the anterior region. Thus, it can be concluded that such high values for the fracture load would provide protection to the tooth not only during masticatory loading but capacitate them for a strong traumatic force also. However, the difference of values between the vertical and horizontal load were not statistically significant which could be because the reinforcement ability of the materials that remained constant and only the direction of force differed.

The direction of the fracture lines was categorized as mesio-distal, bucco-lingual, and multiple fracture. Multiple fractures were recorded highest in each group which could be attributed to very less amount of dentine that remains after the bio-mechanical preparation.[17]

Limitations and recommendations

At present, the evidence is quite scarce to provide any recommendation about the possibility of using any obturating material alternatively to other to increase fracture resistance of primary teeth. Yet, the present research might provide a strong reference for further trials in this field advocating larger sample size and a standardized methodology to eradicate alterations and assortments in the samples. As this was an *in vitro* evaluation, further *in vivo* investigation with newer materials is recommended.

Conclusion

The null hypothesis was not accepted as there was statistically significant difference between endoflas and other obturating materials. It can be concluded that Zinc oxide eugenol, Vitapex and Endoflas play an important role in substituting the lost part of the tooth structure and provide reinforcement to the remaining tooth structure to some extent. From the above discussion, it becomes clear that, there is a continuous need for evaluation of various upcoming obturating materials for primary teeth to make evidence-based decision.

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Not applicable.

Conflicts of interest

There are no conflicts of interest.

Author contributions

TN: Contribution: literature review, experimental/ laboratory/treatment procedures, statistical analysis, manuscript preparation; SD: Contribution: study concept;

SS: Contribution: study design, editing and review; SM: Contribution: editing and review; AN: Contribution: editing and review; JL: Contribution: editing and review.

Ethical policy and institutional review board statement

The present study was an in-vitro comparative study conducted in Department of Pedodontics and Preventive Dentistry, People`s College of Dental Science and Research Centre and Praj Metallurgical Laboratory, Pune for the evaluation of fracture resistance using Instron Universal Testing Machine after obtaining ethical clearance from the institutional review board of People`s University (Ref/PCDS/Acad./12–13/3659 (6). The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standard.

Patient declaration of consent

As a part of In-Vitro study, Study details were explained, and informed consent was obtained from all the patient's parents. The consent form was prepared according to WHO Informed Parental Consent form for Research.

Data availability statement

The data sets investigated during the present study are available from the corresponding author on request.

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