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ORIGINAL ARTICLE

# Invivo Comparative Evaluation of Tertiary Dentin Deposit to Three Different Luting Cements a Histopathological Study

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**Abstract** The aim of the study is to evaluate histopathologically the amount of tertiary dentin deposit stimulated by three different luting cements. With the informed consent for fifteen patients crown preparation was done for maxillary and mandibular premolar teeth which were scheduled for orthodontic extraction. Copings were cemented with three different luting cements zinc oxide eugenol, glass ionomer and zinc polycarboxylate which were classified as Groups A, B and C respectively. The teeth were later extracted and histopathologically analysed for pulpodentinal reactions using a control study group. Statistically Tukey-HSD procedure was used to identify the significant group and one way ANNOVA was used to analyse the thickness of tertiary dentin among the study group. Tertiary dentin was seen in most of the specimens. When the three groups were compared zinc oxide eugenol helps in stimulation of tertiary dentin formation.

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**Keywords** Tertiary dentin · Zinc oxide eugenol · Decalcification

## Introduction

The tooth pulp detects and responds to dentinal injuries resulting from caries, attrition, abrasion, trauma and restorative dental procedures. When 1 mm$^2$ of dentin is exposed, about 30,000 living cells are damaged [1]. So the amount of exposed dentin and cell death is more in relation to a crown preparation compared to a cavity preparation, erosion and abrasion.

The deposits of tertiary dentin matrix are the main pulpal repair response to injurious conditions [2, 3]. Tertiary dentin is secreted locally by odontoblasts as a response to primary and secondary dentinal injuries [2–5]. The process of tertiary dentin secretion can be classified as being reactionary and/or reparative in origin secreted by newly differentiated odontoblast like cells [6].

The aim of the study is to evaluate histopathologically the amount of tertiary dentin deposit stimulated by three different luting cements.

## Materials and Methods

Fifteen dentulous patients falling under the age group of 15–23 were selected, for whom the maxillary first premolar and the mandibular first premolar had been scheduled for orthodontic extraction. Two specimens were collected from each patient, one maxillary premolar and one mandibular premolar.

With the informed consent of the patient and the guardian, the crown preparation under local anaesthesia for a full ceramic crown was prepared using rotary hand piece with water spray, using least possible pressure of a drill speed of 2,00,000 revolutions per minute were done using standardized diamond burs (No. 171 SHOFU) and the margins of the preparation were supra gingivally placed. Care was taken not to damage the surrounding soft tissues. Impressions were made using elastomeric impression material (Zetaplus) and the cast were prepared by pouring die stone (Ultrarock). Later die preparations were done using pin die system and wax patterns were fabricated and invested using phosphate bonded investment material (Bellaves-T). Castings were done with Nickel-Chrome alloy (Densply–Sankin) and the copings were trimmed, finished and polished.

The finished copings were checked inside the patient's mouth for proper fit. Care was taken to check whether the coping was in contact with the occlusal tooth to prevent occlusal load. After satisfactory trial the prepared tooth was isolated for moisture control and the copings were cemented. Out of 30 copings, 10 copings (Group A) were cemented using zinc oxide eugenol cement (DPI, India), (Group B) 10 copings were cemented using glass ionomer cement (FUJI I, Tokyo, Japan), (Group C) 10 copings were cemented with zinc polycarboxylate cement (Poly F, Dentsply, Konstanz, GERMANY).

Later the patients were instructed to report after 30 days. The teeth copings were removed and the teeth were extracted under local anesthesia and sent for histological study.

The extracted tooth was fixed in 10 % neutral buffered formalin for 24 h. Decalcification was done for the extracted teeth using sodium formic acid solution for duration of 3 weeks. After decalcification the tissue is processed and stained with eosin and hematoxylin before mounting using Dysterene plasticizer xylene and viewed under the microscope.

The sections were viewed under low power (10×), under high power (40×) binocular microscope and the thickness of the tertiary dentin were identified and measured using an eyepiece grid [7, 8].

### Control Study Group

With the informed consent of the patient and the guardian, a normal sound tooth (premolar), which was scheduled for orthodontic extraction was removed under local anesthesia and fixed in 10 % neutral formalin for 24 h, which was later demineralized using sodium formic acid solution for a period of 3 weeks, then routinely processed and embedded in paraffin wax. Sections made were 5 micrometer thickness and routinely stained with eosin and hematoxylin. These specimens were used as a control study group to compare with the other study groups (Groups A, B and C) for pulpodentinal reactions.

## Results

Histopathological examination revealed that presence of reactionary or tertiary dentin present in most of the specimens.

The thickness of the tertiary dentin was not found to be uniform. Considering the quality of the reactionary dentin the mineralization was not found to be equal, alternating low and high mineral content were seen and interglobular dentin was frequently observed. The study was under a sample size of 10 in each group tabulated in Table 1.

One way ANNOVA suggested there is significant difference in mean tertiary dentin thickness in the three different study groups as shown in Table 2; Graph 1. However, there is no significant difference in mean thickness of tertiary dentin between Groups B and C ($p > 0.05$) as shown in Table 3; Graphs 2, 3.

Table 4 shows mean standard deviation and test of significance of mean age between different study groups.

Peavson's correlation co-efficient which estimated to assist the linear relationship between age and thickness in each study group showed there is significant negative correlation between age and tertiary dentin thickness in Group B ($p = 0.009$) in Table 5.

Results of linear regression analysis in the three study groups shows age increases by 1 year, the thickness may be decreased by 0.29 mm in Group B as given in Table 6.

**Table 1** Mean and standard deviation of tertiary dentin thickness in different study groups

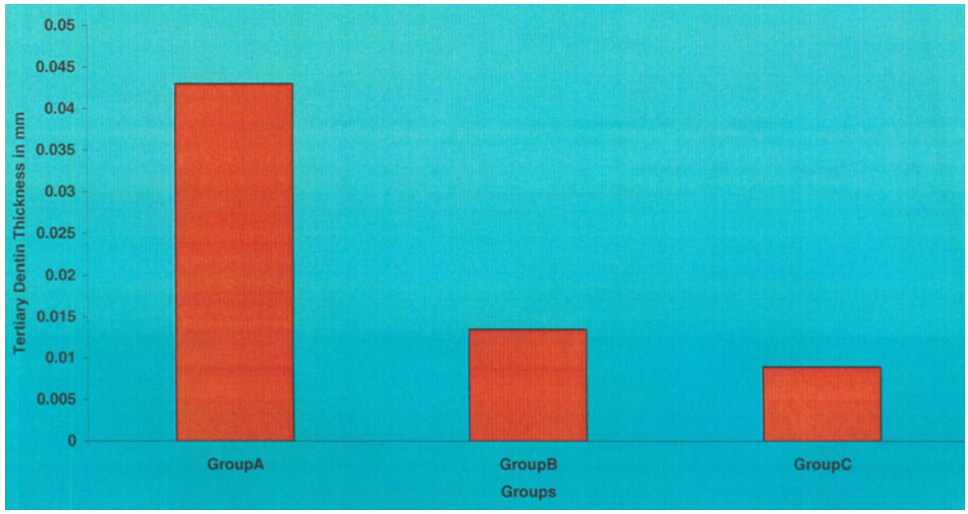
| Study groups | Mean reparative dentin | Standard deviation thickness |
|---|---|---|
| A-zinc oxide eugenol | 0.043 | 0.0134 |
| B-glass ionomer cement | 1.0135 | 0.0100 |
| C-zinc polycarboxylate | 0.0090 | 0.0074 |

Sample size $n = 10$, in each study group

**Table 2** Results of one-way ANOVA to compare the mean thickness of tertiary dentin in different study Groups

| Source of variation | df | Sum of squares | Mean sum of squares | $F$ Ratio | $p$ value |
|---|---|---|---|---|---|
| Between groups | 2 | 0.0068 | 0.0034 | 30.67 | <0.0001/siGma |
| Within groups | 27 | 0.003 | 0.001 | – | – |
| Total | 29 | 0.0098 | – | – | – |

*df* degree of freedom




**Graph 1** Mean tertiary dentin thickness in three different groups

**Table 3** Result of multiple range test by Tukey—HSD procedure to identify the significant group

| Study groups | Zinc oxide eugenol (A) | Glass ionomer (B) | Zinc polycarboxylate (C) |
|---|---|---|---|
| A-zinc oxide eugenol | | | |
| B-glass ionomer | Significant | | |
| C-zinc polycarboxylate | Significant | Non-significant | |

Significant at 5 % level ($p \leq 0.05$)

Non-significant at 5 % level ( $p > 0.05$)

## Discussion

The study was conducted to estimate the amount of tertiary dentin deposit to three different luting cements. After crown preparation coping was made and luted using three different luting cements (Groups A, B and C) and observed for a period of 1 month. Later the teeth were extracted and fixed in buffered formalin, the samples were decalcified, processed and stained with eosin and hematoxylin. The prepared slides were evaluated histopathologically for pulpo-dentinal reaction.

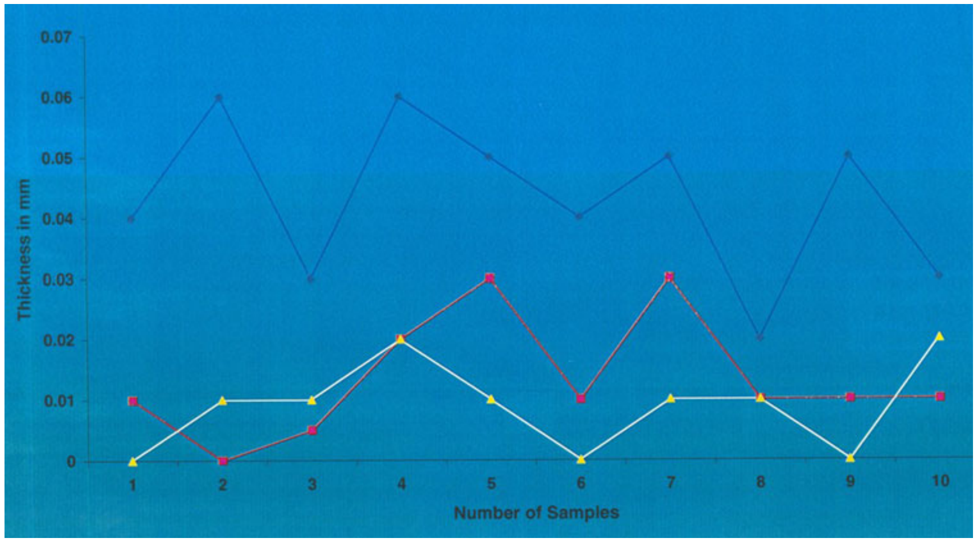
The number of odontoblastic process damaged in relation to crown preparation is comparatively more than the cavity preparation, erosion, abrasion and attrition [1].

The three study groups A-zinc oxide eugenol (DPI, INDIA), B-glass ionomer (FUJI I, Tokyo, JAPAN) C-zinc polycarboxylate, (Poly F, Dentsply Konstanz, GERMANY) were taken to assess the amount of tertiary dentin formation. The calcium hydroxide cement is an ideal material for pulp healing. Due to its poor mechanical property, it is never used as provisional luting cement. Zinc oxide eugenol, one of the finest cement would give a perfect bacterial seal by preventing bacterial entrapment into the dentinal tubules and stimulate tertiary dentin formation. Studies in primates have shown hyperemia, dilatation of vessels followed by pulpal edema and tertiary dentin formation. Comparative studies of zinc oxide eugenol and various cements have shown that it is the most efficient restorative material to prevent microleakage [9–12].

In the study Group B and Group C (glass ionomer and zinc polycarboxylate), the liquid components of the cement consist of polyacrylic acid. The acid molecules are larger than the dentinal tubules. Therefore the amount of permeability is less. The permeability of dentinal tubules plays an important role in stimulation of tertiary dentin. Studies have shown that the permeability of dentinal tubules is more in teeth with necrotic pulp. During crown cementation the chemical ingredients can penetrate through the dentinal tubules and cause pulpal inflammation. When zinc oxide eugenol cement is used, the eugenol which is a pulpal stimulant can penetrate the dentinal tubules and stimulate tertiary dentin formation. Later when the crown is cemented with permanent cement such as glass ionomer cement and zinc polycarboxylate, the chemical agents cannot reach the pulp to cause pulpal inflammation because the tertiary dentin has very minimal or nil amount of permeability which is a protective mechanism of the pulp (Fig. 1). Further studies proved that the tertiary dentin was more amorphous less tubular, and less regular than primary dentin which hinders the passage of copper ion, indicating the tertiary dentin is less permeable as compared with the primary and secondary dentin [13–16].

The quality of tertiary dentin evaluated histopathologically revealed irregularly arranged dentinal tubules (Fig. 2). The thicknesses of the dentin in the three study groups were not uniform. There were different thicknesses of tertiary dentin deposit in a single tooth specimen. It was also observed that there were more of dentinal deposits in the pulpal floor area compared to other areas. Similar

**Graph 2** Thickness of tertiary dentin in three different groups




**Graph 3** Mean thickness of tertiary dentin in different age groups for different study groups

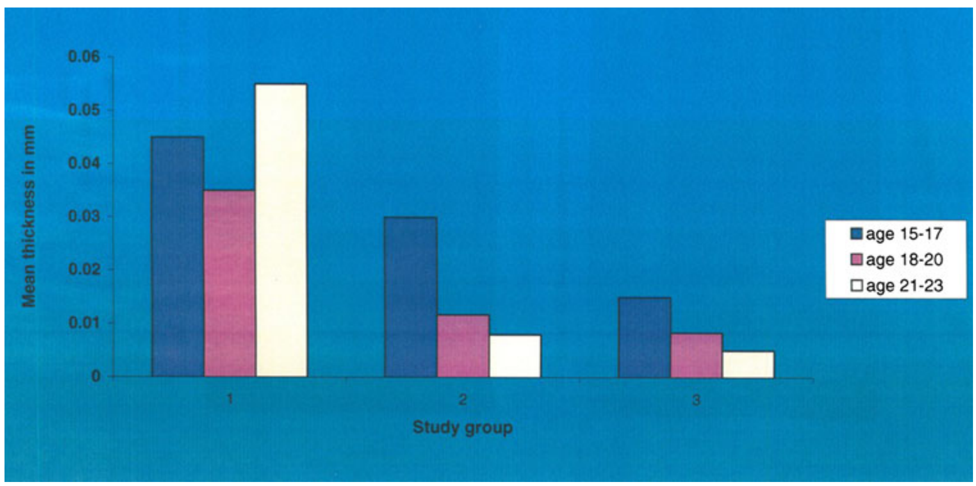



**Table 4** Mean, standard deviation and test of significance of mean age between different study groups

| Study groups | Mean age (years) | SD | *p* value* |
|---|---|---|---|
| A-zinc oxide eugenol | 18.4 | 3.6 | |
| B-glass ionomer | 18.9 | 2.5 | |
| C-zinc polycarboxylate | 18.9 | 1.7 | 0.34 (non-significant) |

*One way ANNOVA was used to calculate the *p* value

**Table 5** Correlation analysis of tertiary dentin thickness and age

| Study groups | Correlation co-efficient | *p* value |
|---|---|---|
| A-zinc oxide eugenol | 0.12 | 0.37 (non-significant) |
| B-glass ionomer | −0.73 | 0.009 (significant) |
| C-zinc polycarboxylate | −0.37 | 0.15 (non-significant) |

observations were seen in studies which reported that irregular tertiary dentin forms more on the floor than on the lateral wall which is stimulated physiologically in response to pulpal injury [17, 18].

The thickness of dentin was found to be more in study Group A (zinc oxide eugenol) (Fig. 2) compared with other two study Group B (glass ionomer cement) (Fig. 3) and Group C (zinc polycarboxylate) (Fig. 4). Studies on the effect of remaining dentin thickness beneath deep cavity on the rate of tertiary dentin formation concluded that, in deep cavities with a remaining dentin thickness of 500 microns there was less amount of tertiary dentin thickness compared with dentin thickness of 700 microns [19].

Considering the daily deposit of dentin approximately 4 microns of the dentin deposited during initial dentinogenesis. After tooth eruption the amount of dentin deposits slows down to 1 micron per day. During the process of

**Table 6** Result of linear regression analysis in different study group dependent variant—tertiary dentin thickness independent variable age

| Study groups | Regression co-efficient | | *p* value |
|---|---|---|---|
| | β | SE (β) | |
| A-zinc oxide eugenol | 0.0006 | 0.0018 | 0.74 non-significant |
| B-glass ionomer | −0.0029 | 0.0010 | 0.02 significant |
| C-zinc polycarboxylate | −0.0016 | 0.0015 | 0.29 non-significant |

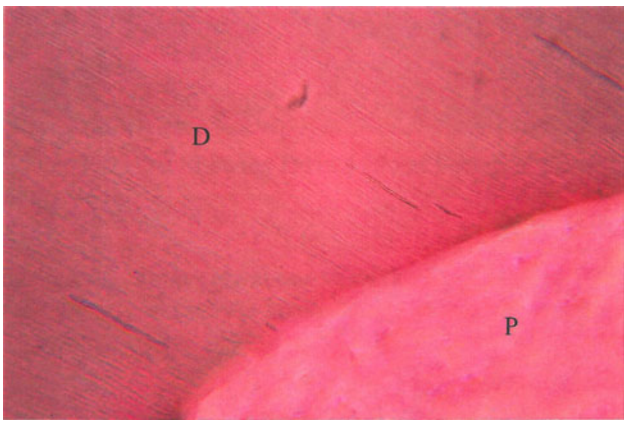



**Fig. 1** Nil amount of tertiary dentin. *D* dentin, *TD* tertiary dentin, *P* pulp

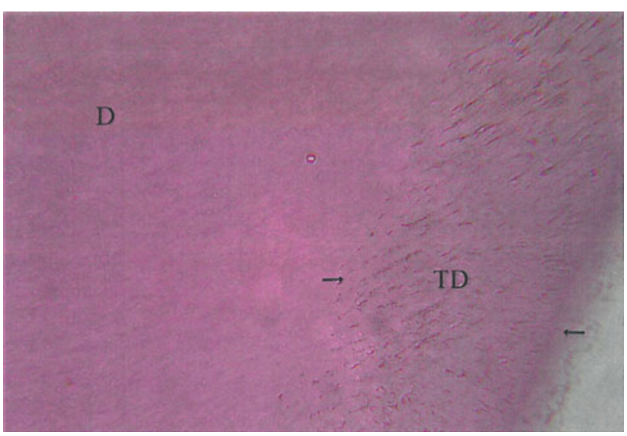



**Fig. 2** Maximum thickness of tertiary dentin in Group A

tertiary dentin formation due to stimulation of pulp response the daily amount of dentin may form at a rate of 4 microns per day [1].

Studies have shown that the rate of formation of tertiary dentin in primates over a period of 119 days and it was observed that in the first 98 days the rate of formation of tertiary dentin was significantly greater than the last 21 days where there was no difference in the daily rate of tertiary dentin deposit beneath the lining material. In the above study the maximum amount of dentinal thickness

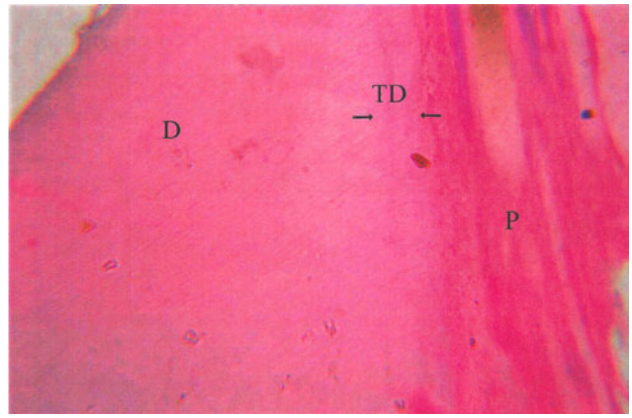



**Fig. 3** Maximum thickness of tertiary dentin in Group B. *D* dentin, *TD* tertiary dentin, *P* pulp

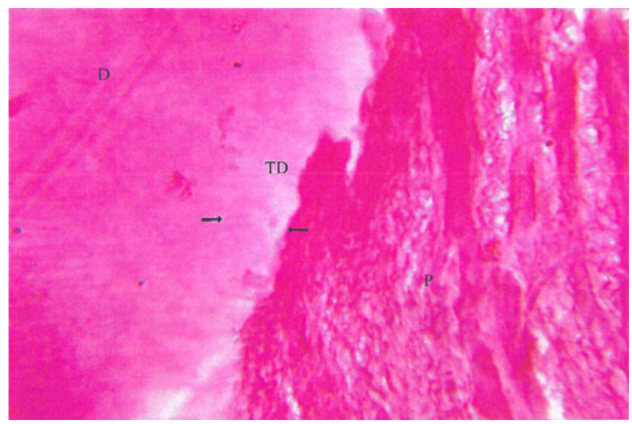



**Fig. 4** Maximum thickness of tertiary dentin in Group C

achieved was 60 microns (Fig. 2) in duration of 30 days. This shows that the results of this study and other similar studies correlate [20].

Taking age and dentin thickness into consideration this study has shown that as age increases the amount of dentin deposit decreases. Studies concluded that the age of the patient, the choice of the tertiary material, and the size of the restoration prepared are the factors that can positively or negatively alter the pulpal repair response. The study also concluded that there is a significant increase in the deposition of tertiary dentin as age increases [21].

The results of the above study in relation to age do not correlate with the other studies conducted by similar approaches. The variation of the results may be due to minimum amount of specimens and the age group taken for the study was restricted from 15 to 23 years. The other related studies have taken more number of specimens and wider age group comparatively.

The pulpal changes in the above study Groups has shown that there is a mild inflammatory response in the entire three groups (Figs. 5, 6). The histopathological study

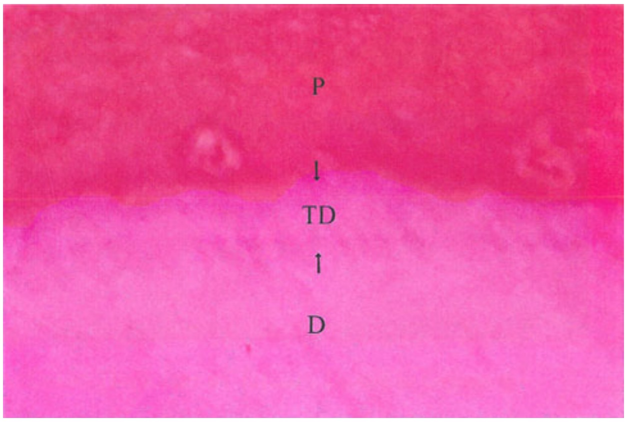



**Fig. 5** Tertiary dentin thickness under ×10 magnification

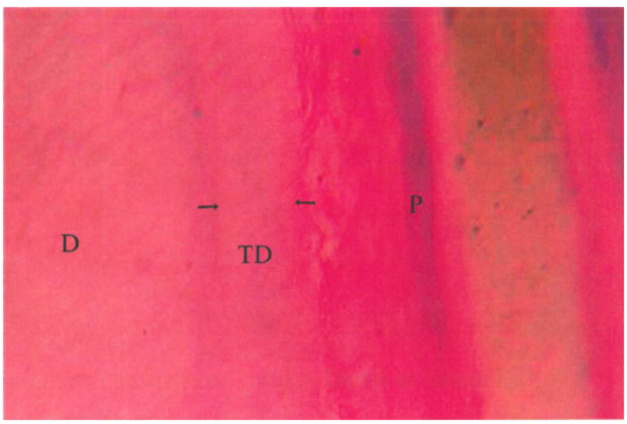



**Fig. 6** Tertiary dentin thickness under ×40 magnification. *D* dentin, *TD* tertiary dentin, *P* pulp

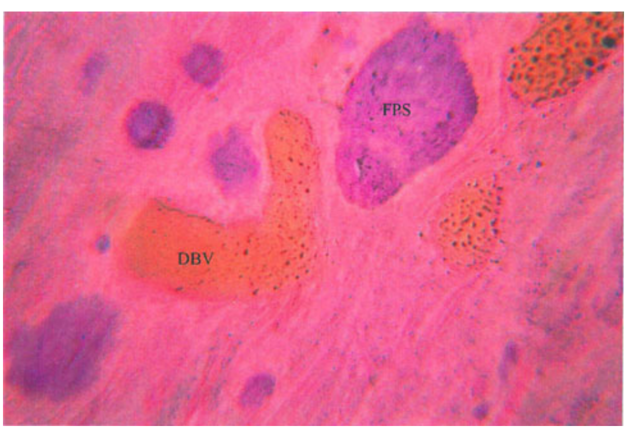



**Fig. 7** Pulpal reaction showing dilated blood vessels & false pulp stone. *DBV* dilated blood vessels, *FPS* false pulp stone

reveals the presence of the dilated and engorged blood vessels. False pulp stones were also demonstrated in two specimens which may be formed due to irritation of pulp (Fig. 7). Studies have demonstrated that while comparing the bio compatibility of responsive materials used in United Kingdom all the cements were considered to be irritant to the pulp. None of the cement had ideal biological property [3, 22, 23].

The observation period of 30 days was considered adequate. The studies in primates demonstrated tertiary dentin formation in 16 of cementation and the amount of tertiary dentin formation was more after 30 days comparatively. In other studies, the observation period was 199 days which showed the amount of tertiary dentin deposit was more in the first 98 days than in the last 21 days [12, 24].

## Conclusion

There have been various studies done to study the amount of tertiary dentin deposits histopathologically in relation to cavity preparation which has been quoted in other journals [9, 13]. Tertiary dentin formation in relation to crown preparation and luting cements has been first of its kind in the present study. The study has been undertaken to determine the amount of tertiary dentin formation to a crown preparation cemented with three different luting agents. (Group A-zinc oxide eugenol, Group B-glass ionomer, Group C-zinc polycarboxylate). The results of the study concluded that there were tertiary dentin deposits in all the three study groups and the amount of tertiary dentin thickness was comparatively more in relation to Group A-zinc oxide eugenol (DPI, India),. The other two groups B—GIC (FUJI I, Tokyo, Japan) and C—zinc polycarboxylate (Poly F, Dentsply, Konstanz, GERMANY) did not show much of significance in the tertiary dentin thickness.

While taking age into consideration, there was a regression in the deposit of the tertiary dentin thickness in the age group of 20–23 compared with the other age groups. Further studies have to be conducted with a wider range of age groups and require more number of samples to analyse the relationship of age compared with tertiary dentin.

According to the study, it is preferable to prolong the temporary cementation for a month or more, so that it can stimulate tertiary dentin formation, which is a physiological protective mechanism of the pulp.

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