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*Original Article*

# Effect of intracanal medicaments on radicular dentine: An attenuated total reflection-Fourier-transform infrared spectroscopy analysis

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## ABSTRACT

**Objective:** This study aims to evaluate the effect of proton-pump inhibitor, triple antibiotic paste, and calcium hydroxide on the chemical arrangement of radicular dentine by Fourier-transform infrared (FTIR) spectroscopy and its effect on dentine matrix of root canal under scanning electron microscopy.

**Materials and Methods:** Eighteen mandibular premolars were sectioned to obtain 72 radicular dentine discs and exposed to triple antibiotic paste (tripaste), proton-pump inhibitor (PPI), calcium hydroxide (CH) paste, and deionized water (control) for 1, 2, or 4 weeks. FTIR analyzes the relative loss of organic and inorganic components using phosphate/amide I ratios of all samples.

**Results:** Data obtained were analyzed using one-way ANOVA and *post hoc* comparisons. There was a significant difference in phosphate/amide I ratios for all the specimen statistically. Higher phosphate/amide I ratio was seen in sample treated with triple antibiotic paste (TAP) for 4 weeks as compared to 1 week ($P = 0.24$) and 2 weeks treated dentine ($P = 0.34$). The phosphate/amide I ratio of 1 week treated dentine was significantly higher than that of 2 weeks treated dentine. Sample treated with PPI for 4 weeks had lower phosphate/amide I ratio as compared to 1 week ($P = 0.08$) and 2 weeks treated dentine ($P = 0.34$), but there was no significant difference in 1 week and 2 weeks treated dentine. There was no significant effect of time for CH-treated dentine or in control group ($P > 0.05$).

**Conclusion:** All medicaments caused demineralization of radicular dentine depending on the duration of time. PPI caused maximum demineralization when treated for longer duration as compared to the use of TAP and CH.

**Keywords:** Calcium hydroxide, Proton-pump inhibitor, Scanning electron microscope, Phosphate amide ratio, Radicular dentine

## INTRODUCTION

Endodontists are in a continuous endeavor for the search of newer techniques, materials, and drugs for the management of various endodontic problems. In this effort, endodontic regeneration has gained popularity in the past few decades with promising results for the management of necrotic immature permanent teeth. The regenerative endodontic technique induces the dentinal pulp complex to increase the thickness of the root canal wall, thus overcoming the possibility of root fracture related with the traditional apexification procedures.[1] One of the primary steps toward the success of an endodontic regeneration is the control of infection which can be achieved by various intracanal medicaments such as chlorhexidine (CHX), calcium hydroxide (CH), triple antibiotic paste (TAP), and double antibiotic paste (DAP).[2,3]

CH successfully sterilizes the root canal, but its long-term use had resulted in root fracture as it affects the chemical and organic protein structure of radicular dentine.[4,5] TAP, an amalgamation of metronidazole, ciprofloxacin, and minocycline,[6,7] provides antimicrobial activity against most of the endodontic pathogens.[8] TAP also affects the mineral content which may lead to reduced microhardness, increased permeability, and demineralization of the root canal dentine.[9,10] There are some shortcoming of this paste-like gain of bacterial resistance, allergic reaction, and crown staining due to the presence of minocycline.[11,12] Recently, PPIs have been used in dentistry as intracanal medicament.[13] It acts on proton pump present in the plasma membrane of *E. faecalis and C. albicans* to reduce their acid secretion and the sensitivity by maintaining the alkaline pH.[14,15] PPIs also exert anti-inflammatory and pro-reparative effects, which further enhances the healing of large periapical lesions.[16]

The available literature showed many studies regarding the assessment of antimicrobial properties of PPI, but there are no studies related to its effect on the chemical composition of root dentine. Thus, the present experiment evaluated the influence of CH, TAP, and PPI on the chemical structure of dentinal root surface. Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) measured the phosphate/amide ratio. Scanning electron microscopy (SEM) measured the surface topography.

## MATERIALS AND METHODS

### Sample Preparation

The present *in vitro* study was conducted in the Department of Conservative Dentistry and Endodontics, King George's Medical University (KGMU) in collaboration with Central Drug Research Institute (CDRI), Lucknow, India. The study design was approved by the Institutional Ethical Committee (Reference Code-81 ECM-IIB Thesis/P4). Eighteen freshly extracted single-rooted human permanent mandibular premolars were collected from the Department of Oral and Maxillofacial Surgery, KGMU, Lucknow. Sample size was determined using the formula $n=2X\ (Z_{\alpha/2}+Z_{\beta})^2\ SD^2/d^2$ {n = sample size per group, SD=assumed standard deviation, d = differences in the mean (effect size), $Z_{\alpha/2}$ = significance level, $Z_{\beta}$ = power of the study}. Assuming 80% power, 5% significance level with 95% confidence interval, the required number of teeth per group is six in each group. Extracted teeth after excluding cracked and carious teeth stored in 0.1% thymol solution at 4°C. Two 4 mm dentine cylinders were obtained after decoronating the tooth horizontally at the cementoenamel junction, from the thickest portion of the root. These cylinders were further divided into four dentine discs, thus making a total of 72 samples of dentine discs. To obtain the flat surface of dentine, the disc was flattened with 500 SiC paper [Figure 1]. All the samples were cleaned using deionized water in ultrasonic cleaner for 15 min.

### Preparation of medicaments

TAP was prepared by mixing 500 mg ciprofloxacin, 400 mg metronidazole, and 100 mg minocycline by removing the coating and crushing all the tablets separately in mortar and pestle. The crushed powder was passed through sieve to obtain fine particles. The ciprofloxacin, metronidazole, and minocycline powders thus obtained were weighed individually in the ratio of 1:1:1, and the mixture was dispensed and mixed with deionized water to prepare a thick paste-like consistency. TAP was prepared by mixing antibiotics powder in 1:1:1 ratio with deionized water. CH paste was prepared by mixing CH powder with deionized water (powder/liquid = 2:1). PPI (omeprazole) paste was prepared with deionized water (powder/liquid = 2:1).

### Laboratory procedure

The dentine discs were randomly divided into three experimental and one control group ($n$ = 18). Each treatment paste was applied on the pulpal surface of dentine disc and placed in a small 2 ml Eppendorf tube (Borosil, India) and stored at 37°C for 1, 2, and 4 weeks. For the group of 2 and 4 weeks, specimens were hydrated with 0.05 ml deionized water. At every given time interval, stored samples were taken out and washed thoroughly with deionized water and ultrasonicated for 15 min to washout treatment paste completely.

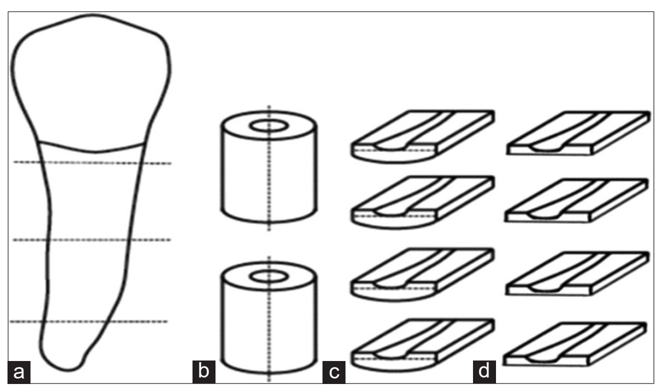


**Figure 1:** Illusration of the method used to obtain dentine discs and the approch used to perform FTIR measurement on the same sample. (a) Dentine discs were cross-sectioned from caries free mandiubular premolar (b) two cylinder of root (c) These cylinder were further divided in to 4 dentine discs (d) The non-pulpal side of each dentine disc was subsequently ground and polished;FTIR measurement and SEM photomicrographs of all treatment groups.

### ATR-FTIR spectroscopy

Spectrophotometer (NICOLET IS5; CDRI, Lucknow, India) with a diamond ATR analyzed the chemical structure of the treated dentine disc. Each dentine specimen was placed on the sample holder of standard FTIR with a diameter opening of 2.5 mm and triplicate spectra were collected from using 70 scans. Spectra Manager CFR software was used to process the obtained spectra by smoothing, baseline correction, and normalization against the amide I peak. The effects of different antibiotics on the chemical structure of dentine were calculated by applying the mineral matrix ratio (it is a ratio of integrated areas of phosphate v1 and v3 peaks to the amide I peak). The final ratio allocated for individual dentine sample denoted the average ratios acquired from the three spectra. Smaller phosphate/amide ratio corresponds to grater dentine demineralization.

### Scanning electron microscopy (SEM)

Four dentine discs were randomly selected from each experimental group for SEM analysis to detect any morphological changes in radicular dentine at 1, 2, and 4 weeks. For SEM analysis, each specimen was sonicated in deionized water for 15 min and dehydrated for 48 h followed by sputter coating with 70 s gold/palladium. Then, specimens were observed under JEOL 7800F scanning electron microscope and images were procured.

### Statistical analysis

The results are presented in mean ± SD. One-way analysis of variance assessed phosphate/amide ratio amid the groups at weeks 1, 2, and 4, followed by Tukey's *post hoc* tests. A paired *t*-test compared the mean change in phosphate/amide ratio from week 1 to 2 and 4. $P < 0.05$ was considered statistically significant. All the analyses were carried out on SPSS 16.0 version (SPSS Inc., Chicago, IL, USA).

## RESULTS

The samples of the control group demonstrated weakening of amide III, amide II, and amide I at 1, 2, and 4 weeks and thus resulting in prominent phosphate and amide peak. As a result, amide and phosphate peaks became more prominent. On the other hand, FTIR spectra indicated the weakening of phosphate and amide peaks in the experimental group at all intervals of time, resulting in apparent amide III, amide II, and amide I peaks [Figure 2].

On statistical analysis, significant difference ($P < 0.05$) in phosphate/amide I ratios amidst all groups at all intervals of time was seen: CH > untreated > TAP > PPI [Table 1]. In TAP group samples, higher phosphate/amide I ratio was observed at 4 weeks than 1 week ($P = 0.24$) and 2 weeks treated dentine ($P = 0.34$). PPI-treated dentine had a lower phosphate/amide

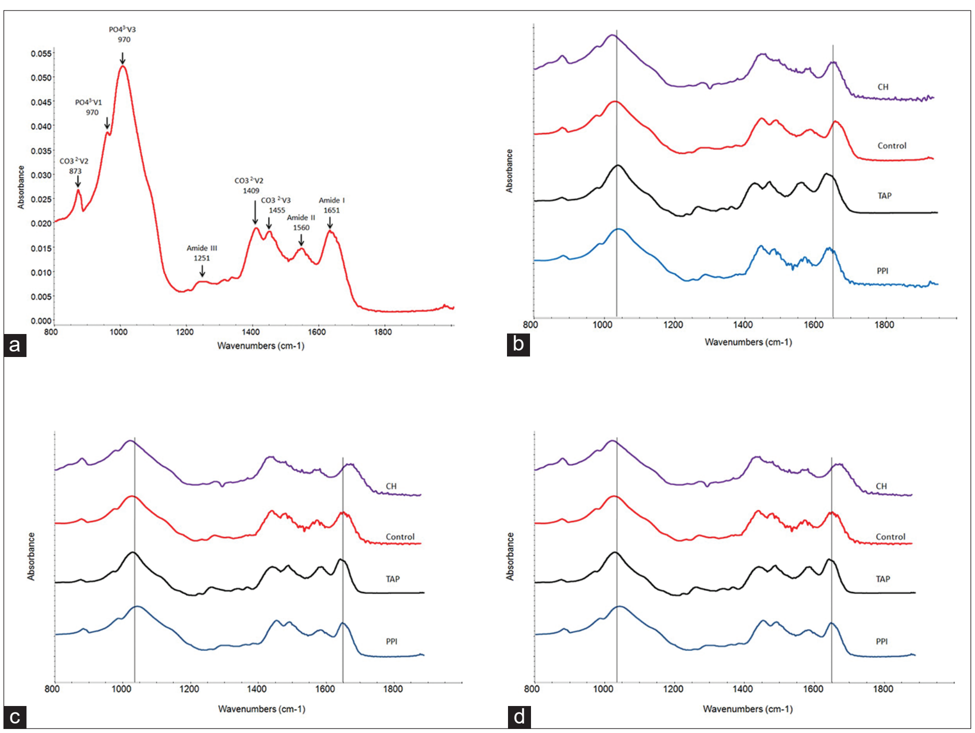


**Figure 2:** Representative ATR spectrum of intact radicular dentine from untreated control group (a), Representative ATR spectra of radicular dentine specimens after exposure to deionized water (control), calcium hydroxide (CH), triple antibiotic paste (TAP), and proton pump inhibitor (PPI), for 1 week (b), 2 weeks (c), and 4 weeks (d).

**Table 1:** Mean (standard error) of the phosphate/amide 1 ratio derived from FTIR for the three treatment groups and the untreated control group

| Groups | Phosphate/amide ratio (Mean±SD) | | |
|---|---|---|---|
| | 1 week | 2 weeks | 4 weeks |
| Control | 4.834±0.447 | 4.764±0.011 | 3.662±1.329 |
| PPI | 3.111±0.087 | 3.197±.080 | 2.5450±.006 |
| TAP | 3.317±0.242 | 2.945±0.344 | 3.813±0.434 |
| CH | 5.281±0.493 | 7.797±5.142 | 8.797±0.329 |
| *P*-value[1] | 0.008* | 0.0001* | 0.41 |

[1]ANOVA test, *Significant. PPI: Proton-pump inhibitor, TAP: Triple antibiotic paste, CH: Calcium hydroxide

I ratio at 4 weeks than 1 week ($P = 0.08$) and 2 weeks treated dentine ($P = 0.34$). There was no significant difference at 1 week and 2 weeks treated dentine.

At lower magnification (×1500), SEM images of the control and CH group showed no sign of demineralization at all intervals of time, whereas TAP group showed little areas of exposed collagen matrix at 1 week and 2 weeks. Samples exposed for 4 weeks showed thin collagen layer which may be due to partial collapse of superficial apatite resulting from over exposure to medicaments. A few areas of exposed collagen matrix could be identified for 2-week and 4-week PPI-treated dentine, which indicates a demineralization effect. However, strong demineralization with intensive collagen exposure was observed when the 4-week PPI-treated dentine was viewed with x5000 [Figure 3].

## DISCUSSION

In endodontic regeneration procedures, minimal instrumentation is required to prevent weakening of the immature root. Thus, there is a key role of disinfection in the treatment of immature necrotic teeth by eradicating endodontic pathogens and to make biological environment conducive for endodontic regeneration.[17] Clinical studies have shown that disinfection initiated by intracanal medicaments not only promotes root development but also leads to increase in thickness of root in the apical region.[18,19] The coronal part of the tooth is more susceptible to fracture close to the cementoenamel junction.[20] Therefore, during endodontic regeneration, disinfectant medicament should possess antimicrobial properties as well as quality to maintain balance between disinfection and its influence on the physical and chemical properties of radicular dentine.

Recently, proton-pump inhibitors (PPIs) have been introduced as intracanal medicaments to eliminate endodontic pathogens and to recuperate the biological environment for endodontic regeneration,[21] but there are no studies regarding the effects of PPIs on chemical properties of radicular dentine.

The concentration of medicament (CH, TAP, and PPIs) used in this study instigated significant demineralization of radicular dentine, which was measured by phosphate/amide ratio. ATR-FTIR evaluated the impact of intracanal drugs on the root canal dentine and the alteration it causes on the chemical configuration of radicular dentine surface.

The higher alkaline pH of calcium hydroxide results in denaturation of dentine organic matrix, which is evident in our study depicted by significant increase in phosphate amide ratio on radicular dentine.[22] Andreason *et al.*[22] in their study demonstrated that frequent cervical fractures of immature teeth treated with CH were related to changes in the organic matrix. The results of the present study justify the previous *in vitro* study that CH has a higher phosphate amide ratio corresponding to a greater dentine collagen deproteinization and less demineralization.[23]

There was a significant reduction in phosphate/amide ratio at 1, 2, and 4 weeks in samples treated with TAP. The acidic nature of TAP (pH=2.9) and the nature of minocycline to bind with calcium are responsible for its ability to demineralize dental hard tissues.[24,25] The significant difference in the phosphate/amide I ratio at the 2$^{nd}$ week and 4$^{th}$ week reveals a gradual rise in the demineralization effect of the TAP with time.[26,27] The lower phosphate amide ratio of TAP compared to the higher phosphate amide ratio of CH indicates that fracture was easier in the CH group. The results of the present study suggested that after 1 and 3 months, there was a significant and continuous decrease in microhardness of dentine on the samples treated with TAP. It also created a significant decline in root resistance to fracture which favors the present study.[28,29] However, during endodontic regeneration, the demineralization effect of the TAP increases stem cells attachment on the surface of radicular dentine through the exposure of collagen fibers.[30] Advanced superficial erosion was observed on SEM analysis in this group which could be explained by the strong acidic nature of TAP.

As evident from Table 1, PPI formulation showed a lower phosphate/amide I ratio on samples treated for 4 weeks than 1 week and 2 weeks treated dentine samples; thus, it shows a gradual increase of demineralization of radicular dentine. PPI showed lower phosphate amide ratio compared to TAP at 1, 2, and 4 weeks because 2 mg/ml showed higher concentration for teeth, which affect the chemical configuration of dentine, but it was not significant. According to an *in vivo* study, the long-term effect of PPI leads to osteoclast formation and the development of osteoporosis at a cellular level leading to the demineralization of bone.[31] SEM photomicrograph [Figure 3d] of PPI showed more open dentinal tubules, indicating increased demineralization as compared to TAP with lower phosphate amide ratio at 1, 2, and 4 weeks.

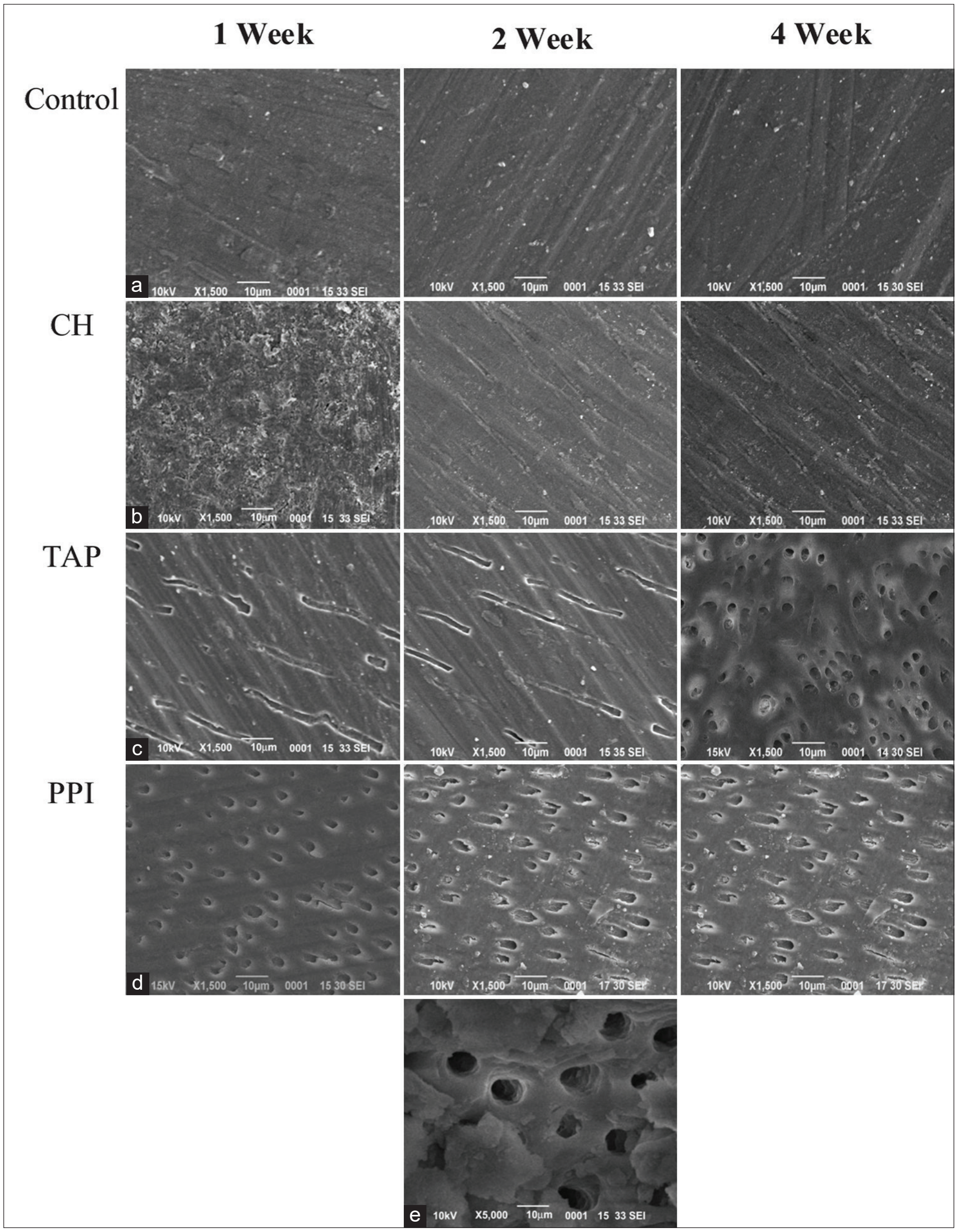


**Figure 3:** SEM photomicrographs of the four groups (a) control groups, (b) calcium hydroxide (CH), (c) Triple antibiotic paste (TAP) at 1500× at 1,2 and 4 weeks, (d) proton pumpinhibitor (PPI) at 1500× at 1,2 and 4 weeks, and (e) PPI-treated dentine at 5000×(high magnification) at 4 weeks.

## CONCLUSION

Within the limitations of this *in vitro* study, it can be stated that among the intracanal medicaments being compared, TAP was least detrimental to the structural integrity of radicular dentine followed by PPI. At the same time, CH was found to have maximum weakening on radicular dentine. PPI being a newer introduction in the field of regenerative endodontic, needs careful use as a longer duration causes more demineralization. Thus, the careful selection of intracanal medicament is warranted. In the future, *in vivo* studies with large number of sample size should be conducted to further validate the results.

### Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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