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A comparative study of effects of Triphala and Tetracycline gel on root conditioning: A scanning electron microscopic study

Dr. Manvi Srivastava
Postgraduate, Department of Periodontics, Bharati Vidyapeeth Dental College, and Hospital
Corresponding author email: manvisrivastava25@gmail.com

Dr. Varsha Rathod
Professor and HOD, Department of Periodontics, Bharati Vidyapeeth Dental College, and Hospital
Email: drvarshavora92@gmail.com

Dr. Palak Dwivedi
Postgraduate, Department of Periodontics, Bharati Vidyapeeth Dental College and Hospital
Email: palak.dwivedi1221@gmail.com

Dr. Antara Parikh
Postgraduate, Department of Periodontics, Bharati Vidyapeeth Dental College and Hospital
Email: antara1231.ap@gmail.com

***Abstract*---**Background: Remnants of dental calculus, contaminated cementum, and subgingival plaque act as a physical barrier between periodontal tissues and root surfaces, which inhibits the formation of new attachment. Treatment of denuded root surface using various chemicals and antimicrobial agents with scaling and root planing helps in active periodontal regeneration. Aim: The aim of this study was to evaluate and compare the demineralizing effects of Triphala and 1% Tetracycline HCl gel on root conditioning using a Scanning Electron Microscope. Settings and design: A total of 50 hemi-sectioned root samples were obtained from 25 extracted, and root planed samples. These were divided into two groups, Group 1: 1% Tetracycline HCl gel and Group 2: Triphala. Materials and method: The two root conditioning agents were applied with cotton pellets on the sectioned roots. The samples were then examined under the scanning electron microscope. Statistical analysis used: Data obtained was compiled on a MS Office Excel Sheet. Inter group

comparison was done using Mann Whitney U test. Comparison of frequencies of categories of variables with groups was done using Chi Square Test. Results: The root conditioning agents used in this study were found to be equally effective in removing the smear layer. Although in terms of uncovering and widening the dentin tubules and unmasking the dentin collagen matrix, tetracycline was statistically more significant. Conclusion: Tetracycline HCl was found to be the better root conditioner among the two agents used.

***Keywords*---**tetracycline hydrochloride, triphala, scanning electron microscope, root surface biomodification.

Introduction

The goal for periodontal therapy is to repair the function of diseased or injured parts of the periodontium by the development and manipulation of their cells, molecules and tissues. The aim of periodontal therapy is to regenerate the lost periodontal attachment which facilitates "New Attachment" by formation of new connective tissue attachment and embedment of new periodontal ligament fibers n to the new cementum which was previously denuded by the periodontal disease.(1) Remnants of dental calculus, contaminated cementum, and subgingival plaque act as a physical barrier between periodontal tissues and root surfaces, which inhibits the formation of new attachment. Treatment of denuded root surface using various chemicals and antimicrobial agents along with SRP helps active periodontal regeneration. (2)

One of the oldest type of periodontal regeneration is by chemically modifying the root surface. In 1883, Marshall was the first to introduce aromatic sulfuric acid into periodontal pockets forthe purpose of root surface modification and decalcification to create an area that is compatible for connective tissue attachment.(3) The rationale behind the use of acid was the microscopic evidence of hyper mineralisation of diseased roots with obliteration of lacunae of cellular cementum by calcific deposits. (4) A variety of chemical root conditioning agents such as citric acid, Tetracycline Hydrochloride (HCl), fibronectin, Ethylenediaminetetraacetic acid (EDTA), Cohn's factor has been tried to enhance new attachment post root instrumentation. (5,6)

Tetracyclines are the derivatives of the polycyclic naphthalene carboxamide. They are broad- spectrum antibiotics which are effective in controlling periodontal pathogens. Tetracycline HCl, Doxycycline HCl and Minocycline have been used as root conditioning agents to demineralize the root surface as it binds strongly to the root surface and can be released in an active form over extended periods of time. Tetracyclines have a low pH in concentrated solution, and this can act as a calcium chelator resulting in demineralization. It inhibits tissue collagenase production and bone resorption. It is known to adsorb to and subsequently desorb from the dentin. It also exposes the collagen matrix and uncovers and widens the orifice of dentinal tubules. A matrix is thereby

provided supporting migration and proliferation of periodontal cells required for periodontal wound healing. It has also been found to be effective for removing smear layer. (1)

It has been believed since ancient times that nature holds all the solutions to problems faced by mankind; such is the medicine derived from plants or animals. Triphala is a recognized polyherbal medicine, equiproportional mixture of fruits of three following medicinal herbs.(7)

1. Amalaki- Phyllanthus emblica
2. Haritaki- (Terminalia chebula)
3. Bibhitaki- (Terminalia bellirica)

Triphala formulation possesses anti-mutagenetic, radio-protecting and antioxidant activity. Triphala decoctions exert broad-spectrum antimicrobial action against antibiotic resistant bacteria isolated from human subjects. (8,9) Triphala has been proved valuable in the prevention and treatment of several disease of the mouth such as spongy and bleeding gums, gingivitis, stomatitis, and dental caries. It helps in prevention of plaque formation on the tooth surface, since it inhibits the sucrose-induced adherence and the glucan-induced aggregation. It has the ability to inhibit the growth and accumulation of Streptococcus mutans (S. mutans) on thesurface of the tooth, preventing the accumulation of acids on the surface of the tooth and thus the demineralization of the tooth surface.(10)

To the best of our knowledge, Triphala has yet not been evaluated to be used as root surface biomodification agent. Hence, in the present study we decided to compare triphala with 1% tetracycline HCl as root surface biomodification agent using scanning electron microscope.

Materials and Method

For the present study 25 freshly extracted permanent human teeth were selected. Samples were selected based on the inclusion & exclusion criteria. Permanent maxillary and mandibular single rooted human teeth, human permanent teeth which were periodontally compromised and human permanent teeth with supra- and sub-gingival calculus were included in the study. Whereas carious teeth, teeth with root surface caries and restoration and non-vital teeth were excluded from the study.

Preparation of agents:

1% Tetracycline HCl gel Tetracycline HCl : 1gm Carbopol 974: 1gm
Water: 100gm

- 98gm of water was used to dissolve the tetracycline HCl.

- Carbopol was added and allowed to disperse, ensuring no lumps were formed.
- It was gently stirred using a glass rod without letting the air bubbles get entrapped.
- The pH 7.0 was reached.

Triphala

Triphala extract preparation:

1. 25 grams of Triphala powder boiled with 200ml water and reduce it to 50ml.
2. The decoction was filtered with fine cloth/sieve and boiled again till it became semisolid consistency.
3. This extract was dried under shade till moisture completely evaporated.
4. Final product was stored in airtight container.

Triphala decoction preparation:

25 grams of Triphala boiled with 200ml water and reduce it to 50 ml.

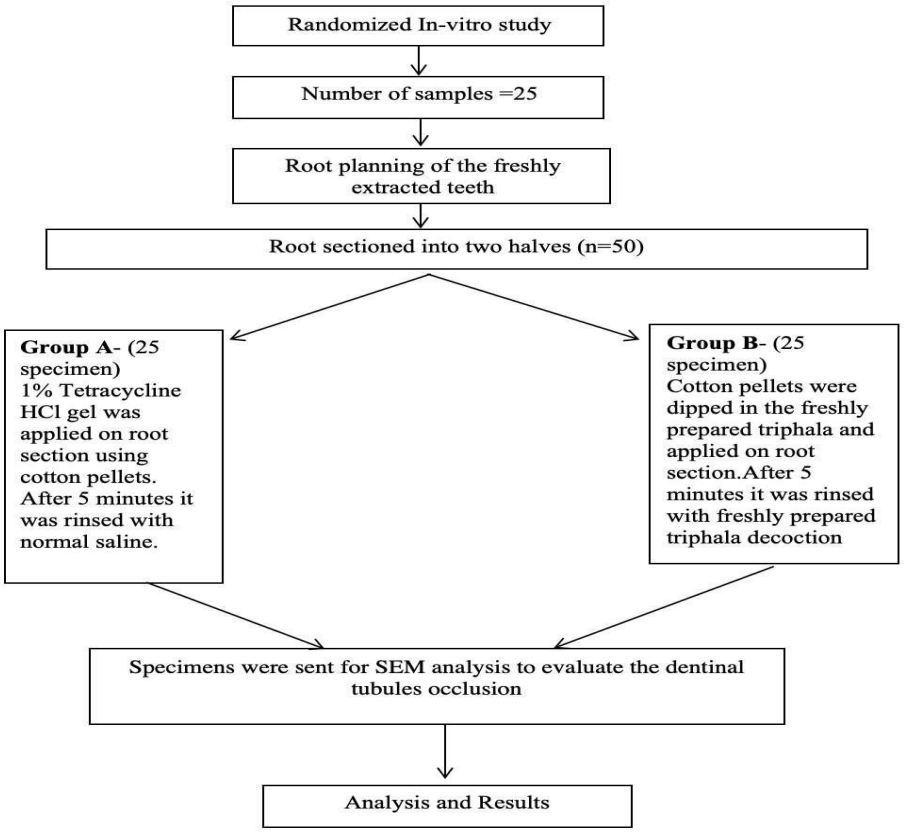


Figure 1: Study Design

Preparation of samples:

1. The root surfaces of 25 freshly extracted teeth were thoroughly planed using Gracey Curette to remove debris, subgingival calculus, and the diseased cementum.
2. The root surfaces of each selected teeth were sectioned longitudinally from the centre using a diamond disc and micromotor under copious water irrigation. Two halves of each specimen yielded us a total number of 50 specimens.
3. The specimens were randomly and equally assigned to the two groups, Group1: 1% tetracycline HCl and Group 2: Triphala.

Application of root conditioning agents:

Group 1: **1% Tetracycline HCl gel**

Cotton pellet was dipped in the 1% tetracycline gel and applied on the sectioned root surface and kept aside for 5mins. (Figure 2)

Group 2: **Triphala**.

Cotton pellet was dipped in the freshly prepared triphala and applied over the root and kept aside for 5 minutes. (Figure 2)
After 5 minutes:

Group 1 sections were rinsed with normal saline
Group 2 sections were rinsed with Triphala decoction

Specimens were sent immediately to SAIF (Sophisticated Analytical Instrument Facility) at IIT BOMBAY for SEM at 3000x magnification (Figure 3), where they were evaluated for the effects of both 1% Tetracycline gel and Triphala on the basis of:

1. Presence or absence of smear layer under SEM
2. Number of exposed dentinal tubules under SEM
3. Total surface area occupied by tubule orifices under SEM

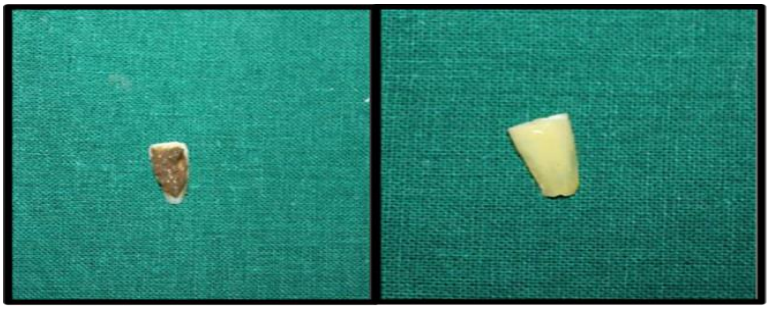


Figure 2: Applying 1% tetracycline HCl gel (Group 1) and triphala (Group 2) on root surfaces

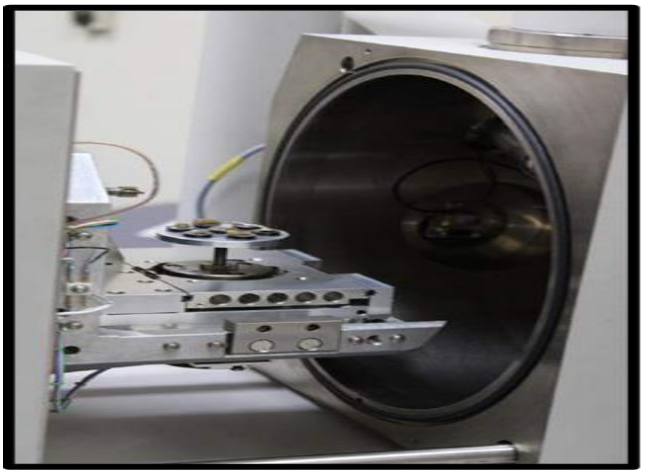
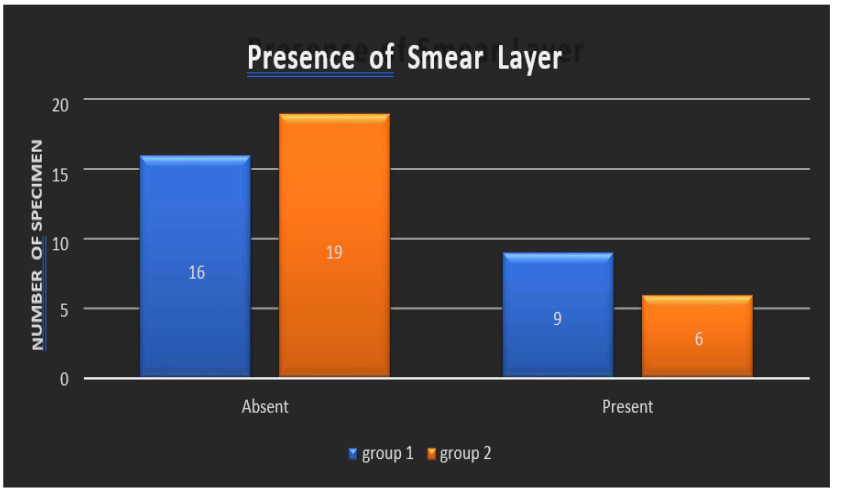


Figure 3 : Placement of the samples in SEM

Results

On examining the microphotographs, the specimens appeared similar and most of the dentinal tubules in both the treatment groups were patent. Image J software was used to determine the number of tubule openings, presence of smear layer and total surface area occupied by tubule orifices. In Group1 (1% tetracycline HCl) out of 25 specimens ,16 specimens showed complete obliteration of the smear layer. In Group 2 (Triphala) out of 25 specimens ,19 specimens also showed absence of smear layer (Figure 4). On performing the Chi square test, it was found that, there was a statistically non-significant difference (p value 0.355) seen between the groups (p>0.05).



Graph 1: Presence or absence of smear layer

	Group				
Smear layer	1	2	Total	Chi-Square value	p value of Chi-Square test
Absent	16	19	35		
Present	9	6	15	0.857	0.355
Total specimens	25	25	50		

Table 1: Presence or Absence of smear layer

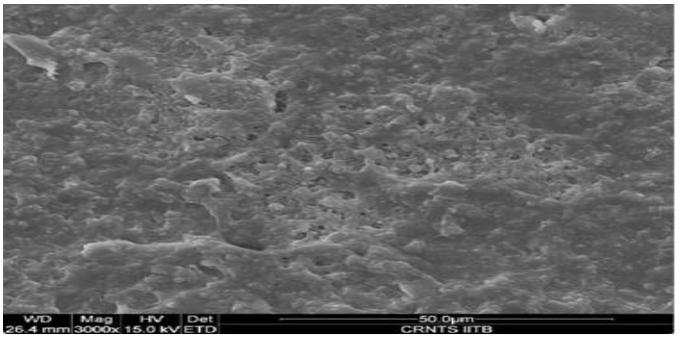
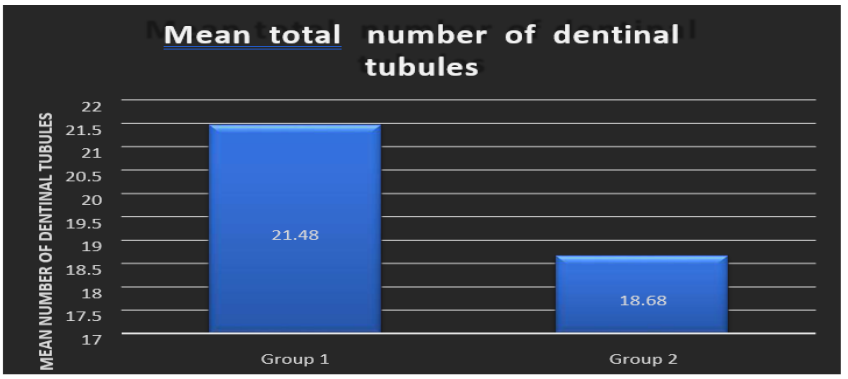


Figure 4 : SEM micrographs showing absence of smear layer

The mean number of dentinal tubules were analysed by the Mann-Whitney U test. In Group 1, the mean number of dentinal tubules were 21.48 with a standard deviation of 4.417 and in Group 2 the mean number of dentinal tubules were 18.68, with a standard deviation of 4.571. There was a statistically significant difference seen in mean number of dentinal tubules between the two groups ($p<0.05$). The mean total dentinal tubules in Group 1 was more as compared to Group 2. (Figure 5)



Graph 2: Mean total number of dentinal tubules

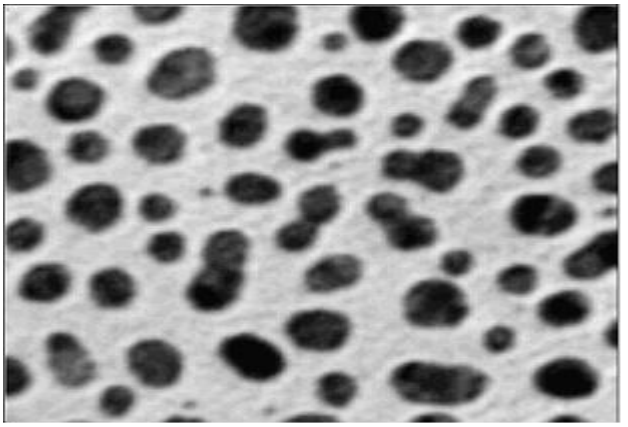
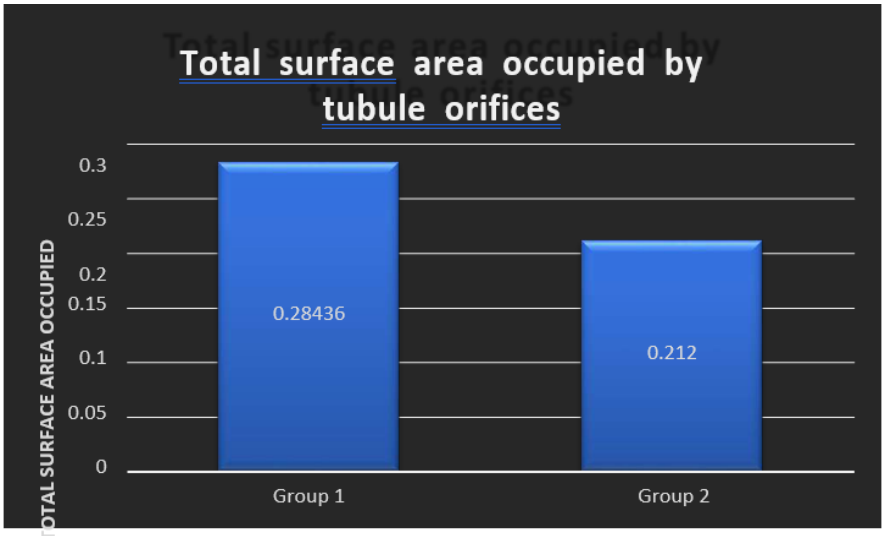


Figure 5: Representative image showing open orifices of dentinal tubules



Graph 3: Total surface area occupied by tubule orifices

Total surface area occupied by tubule orifices which was compared amongst the groups using Mann-Whitney U test. The mean of total surface area occupied by tubule orifices in Group 1 was 0.28 μm^2 and in Group 2 it was 0.21 μm^2. A statistically higher significant difference was seen for the mean of total surface area occupied by tubule orifices between the two groups. The total surface area in Group 1 was more as compared to Group 2. (Figure 6)

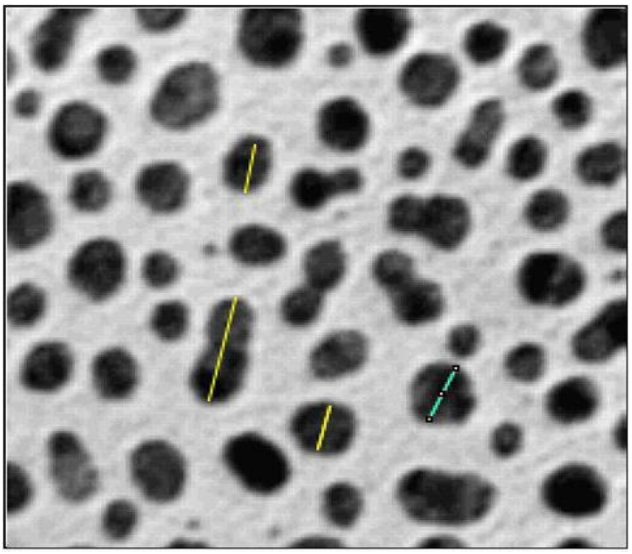


Figure 6: Representative image of calculating diameter (μm) of the open dentinal orifices

Discussion

Periodontal diseases cause pathologic modifications of the healthy periodontal attachment, histologically, physically, and immunochemically. Root surfaces affected by periodontitis exhibit contamination by bacteria and endotoxins and disruption, loss of collagen fibers. [11] These diseased root surfaces become poor foundation for new attachment and proliferation of fibroblasts. Though it has a multifactorial pathogenesis, the most common etiological factor is the bacterial plaque. [12] The root surface modification agents facilitate periodontal regeneration by effectively removing the smear layer formed during scaling and root planing, removal of cementum-bound endotoxin and exposure of collagen from the root. [13] Demineralization of a diseased root is an important step towards regeneration. Since decades chelating agents or acid substances are used to remove the smear layer and to expose root collagen fibers. (14)

Vanheusden AJ et al in their study suggested that citric acid, tetracycline, and its derivatives such as minocycline HCl, doxycycline HCl, are frequently used as root conditioners. The acid pH partially demineralizes the planed root surfaces by eliminating the smear layer which causes opening and widening of the dentin tubules, and exposing various components like Type I collagen, proteoglycans, fibronectin and growth factors of cementum extracellular matrix or dentin extracellular matrix. [15] Wikesjo et al. found that tetracycline HCl which acts in an acidic pH (1.8) when used for surface demineralization enhances binding of matrix proteins to dentinal collagen matrix and stimulates fibroblast attachment and growth. Studies conductedby Daryabegi P et al and Urist

concluded that dentin possess inductive proteins post acid demineralization.[6,16]

Long-term therapy with tetracycline has been shown to have a few disadvantages such as staining of the teeth.[5] Studies suggest that herbal extracts like triphala, when used to treat periodontal disease produce no side-effects. Triphala against PMN-type collagenase (MMP-9) has a strong inhibitory activity. [17] Triphala possesses a few active ingredients of phenolic nature, which are responsible for scavenging free radicals. This is the reason that Triphala has a strong antioxidant activity. Triphala when used as a mouthwash showed results comparable to those obtained by chlorhexidine in their anti-microbial efficacy. [18]

To the best of our knowledge, triphala has not been used as a root surface modifying agent on periodontally affected teeth. In present study, we used triphala as a root surface biomodifying agent on sectioned root surfaces and compared it with 1%Tetracycline HCl which is a proven root surface biomodifying agent. Both the agents were used to assess their efficacy on root surfaces. The parameters checked were, removal of smear layer, number of dentinal tubules seen and area of the open dentinal orifices.

Lasho, O'Leary and Kafrawy in their study [19] used SEM to analyse the conditioned root surface. On examination it was revealed that removal of smear layer by tetracycline HCl (250mg/ml) was better than doxycycline (100mg/ml). In our study too we used SEM to visualize the root surfaces treated with 1% tetracycline HCl and triphala to assess removal of smear layer, presence of number of dentinal tubules seen and surface area of the open dentinal tubule orifices. A smear layer is formed during scaling and root planing due to incomplete removal and translocation of cementum by instrumentation. This smear layer acts as a barrier for the new attachment between the cementum and periodontal cells.[12] Apart from opening of dentinal tubules, root modifying agents like, citric acid or tetracycline-HCl, EDTA, when used primarily aids in eliminating the smear layer.[14] In our study too, when we treated the root surfaces with 1% tetracycline HCl and triphala, it was observed that tetracycline HCl and triphala showed similar effects in removing the smear layer. Study done by Shetty and Dinesh suggested that pH of citric acid (pH 1) and tetracycline (pH

1.6) was acidic enough to expose considerable number of dentinal tubules. Tetracycline treated specimens had the maximum number of dentinal tubules when compared to citric acid and doxycycline.[14,20] Conflicting to these results Misra R et al (1999) in their study reported thatcitric acid solution (pH 1.6) was more effective than tetracycline (150mg/ml) in demineralizing dentin which could be attributed to the lesser concentration of tetracycline used. [21]

Chahal GS, Chhabra V in their study concluded that diameter of specimens treated by tetracycline HCl was significantly higher than those observed in the specimens treated by citric acid and doxycycline. This enlargement or widening of the tubule orifice can be attributed to the preferential demineralization of the peritubular dentin by these agents. (12) In our present study on examining the SEM micrographs, we evaluated and compered the diameter of the dentinal tubule orifices. The area of the dentinal tubule orifices was calculated using the diameter. It was found that the specimens treated by 1% tetracycline HCl had a significantly higher area of dentinal tubule orifices than those treated with triphala.

The present study is an in vitro study with a limited sample size, the findings cannot be anticipated directly to an in vivo situation. The definitive way to determine the clinical efficacy of root biomodification is by conducting randomized controlled trials in a large population and with long term follow up.

Conclusion

Regardless of the debate surrounding the effectiveness of the root biomodification on periodontal regeneration, it is known that the wound healing is better over a planed root surface devoid of any debris. Various agents have been used on the root surfaces to modify the diseased root structure. The root conditioning agents used in this study were found effective in removing smear layer and widening the dentinal tubules. The diameter of the widened dentinal tubules were used to calculate the area of the widened dentinal tubules. To conclude, 1%tetracycline HCl proved to be a better root surface modifying agent in terms of widening of the dentinal tubules when compared to triphala. Though triphala was at par in terms of removal of smear layer when compared with 1% tetracycline HCl.

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