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Comparative Evaluation of Effect of *Aloe Vera* Gel (*Aloe Barbadensis Miller*) on Dentine Root Surface of Periodontally Involved Human Teeth: An Invitro Scanning Electron Microscopic Study
Running title: Effect of *Aloe vera* Gel on Dentine Root Surface

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Abstract: Periodontitis is characterised by inflammation of various components of the periodontium and produces substantial changes in the tooth and root surface which is referred to as a pathologically exposed root surface. As it prevents the creation of new attachments, the smear layer serves as a physical barrier between the root surfaces and periodontal tissues. Although the medicinal use of *Aloe Vera* has been reported, less literature is available regarding its use as root conditioning agent. The purpose of this study was to assess how *Aloe Vera* gel affected periodontally infected root surfaces. Thirty human teeth extracted because of chronic periodontitis were collected. Water and a soft-bristled brush were used to remove blood and saliva from the extracted teeth. The samples were categorized into test group (group 1) - *Aloe Vera* gel and control group (group 0)-placebo gel. Samples were prepared for histological analysis using Scanning Electron Microscopy (SEM). Thirty human teeth extracted because of chronic periodontitis were collected. Water and a soft-bristled brush were used to remove blood and saliva from the extracted teeth. The samples were categorized into test group (group 1) - *Aloe Vera* gel and control group (group 0)-placebo gel. Samples were prepared for histological analysis using Scanning Electron Microscopy (SEM). The number of patent dentinal tubules relative to the total number of dentinal tubules present was evaluated, as was the effectiveness of removing the smear layer. In comparison to the control group (0), the test group's smear layer efficacy was the highest (1.87). The highest number of patent dentinal tubules was seen in test group (5.13) than the control group (0). The present in vitro study concludes that *Aloe Vera* gel as a root conditioning agent to be more efficient in smear layer removal and exposure of dentinal tubules than placebo gel.

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Key Words: *Aloe Vera* Gel, Dentinal Tubules, Smear Layer, Scanning Electron Microscopy, Patent Dentinal Tubules

1. INTRODUCTION

Inflammation of the periodontal structures is a characteristic of periodontitis, which also results in significant alterations to the root and tooth surface, often stated as pathologically exposed tooth root surface. In this case, plaque-induced inflammation destroys the intrinsic and extrinsic fibers, causing the downward proliferation of junctional epithelium. As calculus, plaque and other cytotoxic components breach the root surface which is pathologically exposed; it inhibits new attachment and provides a substrate for bacterial development by creating a physical barrier [1].

As endotoxins have adhered to the diseased root surfaces, cell attachment is not supported [2]. Numerous changes are seen in the exposed cementum, including the accumulation of calculus and plaque, surfaces that are either unmineralized or hypermineralized, endotoxin and cytotoxin contamination, and the lack of collagen cross banding. These modifications inhibit new attachment by reducing fibroblast viability and cell proliferation [3, 4].

The goal of periodontal therapy is to regenerate the supporting tissue on periodontitis-affected tooth surfaces [5]. Plaque and calculus, root-bound toxins, and contaminated cementum are mechanically removed in order to achieve this and furthermore, the denuded root surface is treated with a variety of chemicals and antimicrobials [6-8].

A smear layer made up of remnants of calculus, subgingival plaque, and contaminated cementum will enclose the mechanically debrided root surface making it impossible to fully disinfect the root surface using only mechanical methods as bacterial toxins cannot be eliminated completely [9].As the smear layer hinders the formation of new attachment, it creates as a barrier between the tooth root surfaces and the periodontal tissues.[4]

In addition to root instrumentation, physical techniques such as LASER and a variety of chemical root-conditioning agents (such as Doxycycline Hydrochloride (HCL), Citric Acid, Phosphoric Acid, Minocycline HCL, Fibronectin, Tetracycline HCL, Ethylenediamine tetra acetic Acid (EDTA), Sodium Deoxycholate, Hydrochloric Acid, Cohn's Factor etc.) have been considered as an adjunct [10]. They have also been demonstrated to remove any remaining toxins that are adhering to the diseased root surfaces and to expose proteins attached to cementum and dentin collagen [10] and offer a substrate that facilitates the migration, chemotaxis, and attachment of the cells that takes part in the wound healing and new connective tissue attachment growth [11].

Due to the cost effectiveness and fewer side effects, a variety of herbal and natural materials have been utilized for chemotherapeutic purposes in dentistry and medicine since ancient times [12]. One of the most widely used medicinal plant species with a long history of healing is *Aloe Vera*. It belongs to the Liliaceae family and is one such medicinal perennial succulent plant which has anti-bacterial, anti-fungal, anti-inflammatory, anti-viral, anti-oxidant and immune-stimulating qualities [13].

Two distinct components constitute an *Aloe Vera* leaf: the peripheral bundle sheath cells and its central mucilaginous portion. *Aloe Vera* gel is a thin, clear, tasteless, jelly- like material and is produced by the parenchymal tissue that makes up the central part of *Aloe* leaves. The mucilaginous tissue found in the centre of *Aloe Vera* leaves is used to make *Aloe Vera* gel, a component in a variety of cosmetic and medicinal goods, in recent years. It contains a glycoprotein with cell proliferating and promoting activity. *Aloe Vera* has been demonstrated to significantly promote wound healing following periodontal surgery [14].

Although the medicinal use of *Aloe Vera* has been reported, less literature is available regarding its use as root conditioning agent. Thus, this work attempts to compare the alterations in the surface of planed

root of the tooth following the Aloe Vera gel application using scanning electron microscopy (SEM) analysis.

2. MATERIALS AND METHODS

2.1 Study Protocol

This in-vitro study was carried out in the Periodontology Department, Rajarajeswari Dental College and Hospital, Karnataka, India and Indian Institute of Science, Bangalore, India. For the current investigation, thirty teeth that required extraction due to severe periodontal diseases were obtained. Inclusion and exclusion criteria of the study is explained below.

2.2 Inclusion Criteria

1. No records of any periodontal treatment in the past six months
2. No histories of acute pain or swelling prior to their extraction
3. Teeth devoid of any developmental anomalies
4. Root without any abrasion or erosion

2.3 Exclusion Criteria

1. Teeth that are endodontically treated.
2. Hypoplastic teeth
3. Fractured teeth

2.4 Preparation of the Specimen

A total of 10 extracted teeth due to severe periodontal diseases were collected. All the samples were stored in 10% Formalin at room temperature. A soft bristle brush and distilled water were used to remove the blood clot and saliva. Gracey Curette was used to carefully debride the root surfaces. To achieve an even, glass-like hard surface, the altered cementum is subsequently removed using a finishing bur (no 102R) in a high-speed hand piece at a speed of about 4,00,000 rpm. A section of the root that was 5 mm from the cementoenamel junction (CEJ) was chosen after the crown of each tooth was removed to create the experimental surface (Figure 1). The specimen was cut into two longitudinal sections of equal dimensions along the tooth pulp using a diamond disc that is double sided (Figure 2) set in a hand piece in a slow speed under continuous and ample irrigation. Later, exposing the underlying dentine, the cementum was removed from the root surface using a high-speed Fissure Bur (Figure 3). From each root, three dentin blocks of approximately 4mm x 3mm x 1mm were prepared, for a total of 30 dentin blocks.

Figure 1: Materials Used

Figure 2: Diamond Disc Bur

Figure 3: Fissure Bur

2.5 Preparation of Aloe Vera and Placebo Gel

The following method was used to create two percent *Aloe Vera* gel. Carbopol-940, immersed overnight in decontaminated water containing tissue homogenizer Hydroxypropyl Methyl Cellulose

(HPMC) solution and 0.2% w/v sodium benzoate, was made to mix with propylene glycol. 2 ml aloe vera extract (Supercritical fluid extract, Sami labs, Bengaluru) was added to HPMC solution and mixed thoroughly to make a uniform mixture. This mixture was then transferred to the carbopol solution for further homogenization. For pH neutralisation, triethanolamine was added (Figure 4). The same process was used to make the placebo gel (Figure 5), which was then kept at ambient temperature [14]. The gel was prepared from St. Johns Pharmacy College, Bangalore.

Figure 4: Aloe Vera Gel

Figure 5: Placebo Gel

2.6 Application of Gel

Based on the type of gel used, the prepared teeth specimen was categorized into control group and test group with 15 samples in each group.

Cotton pellets that had been fully soaked in placebo gel were applied to the sample for a total of five minutes in order to treat the control group. Every 30 seconds, these pellets were changed.

Cotton pellets that had been thoroughly soaked in *Aloe Vera* gel were applied to the sample in the test group for a total of five minutes; the pellets were changed every thirty seconds.

2.7 SEM Analysis

The analysis was conducted in Indian Institute of Science, Bangalore. The samples were processed by coating it with gold so that it increases the conductivity of electrons and sticked on to aluminium stub (Figure 6) and placed inside the Scanning Electron Microscope (ThermoFisher® Apreo 2S HiVac) (Figure 7) and analysis was done in Indian Institute of Science, Bangalore. The samples were observed at 1500x to 4000x magnification.

Figure 6: Stab

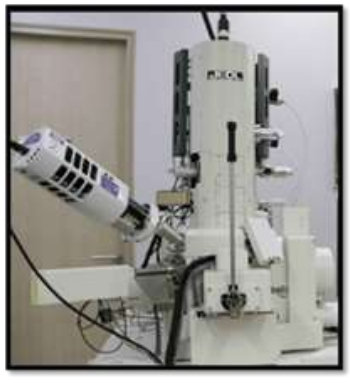

Figure 7: Scanning Electron Microscope

The recorded micrographs were assessed for:

- Effectiveness of removal of smear layer

Removal of smear layer was assessed by Madison and Hokett scale given in the year 1997 based on the following criteria; score 0: No removal or no apparent effect on the smear layer, score 1: Greater than no effect, but less than one-half removal, score 2: Approximately one-half removal of the smear layer,

score 3: Greater than one-half but less than complete removal, score 4: Complete removal of the smear layer with clean and open dentinal tubules [15].

- Number of patent dentinal tubules by that of the number of total dentinal tubules present.

2.8 Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. Descriptive analysis includes expression of Dentin Tubules in terms of Mean & SD for continuous variables. Mann Whitney Test was used to compare the mean dentin tubules & patent tubules between Control & *Aloe Vera* group. Wilcoxon Signed Rank test was used to compare the mean overall dentin & patent tubules in the *Aloe Vera* group. The level of significance was set at $P<0.05$.

3. RESULTS

The SEM analysis revealed that specimen treated with placebo gel showed no smear layer removal and dentinal tubule exposures (Figure 8). And the specimen treated with *Aloe Vera* group showed dentinal tubule exposure and partial removal of smear layer removal and amongst which few of them were patent. (Figure 9 and 10).

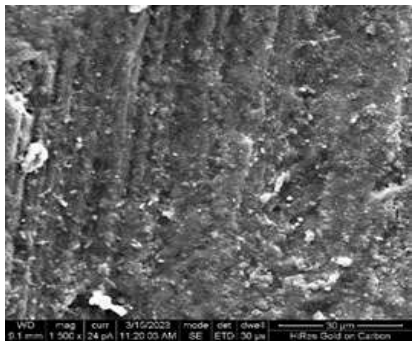

Figure 8: SEM Image of Specimen Treated with Placebo Gel

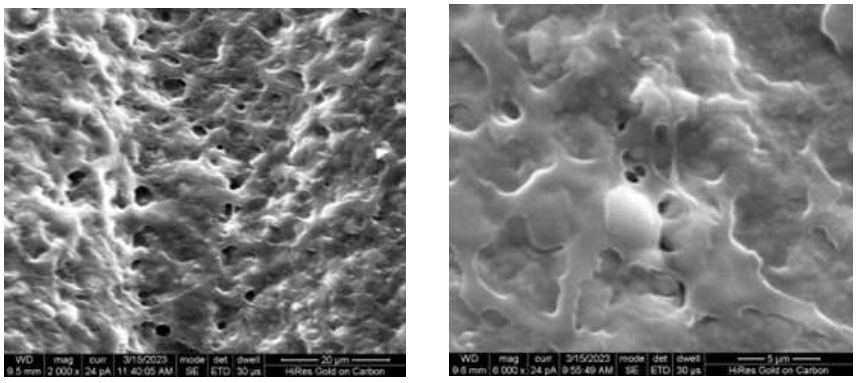

Figure 9 & 10: SEM Image of Specimen Treated with Aloe Vera Gel.

The mean number of dentin tubules in the *Aloe Vera* group was significantly higher as compared to control group and the mean difference was statistically significant at $p<0.001$ (Table 1 and graph 1).

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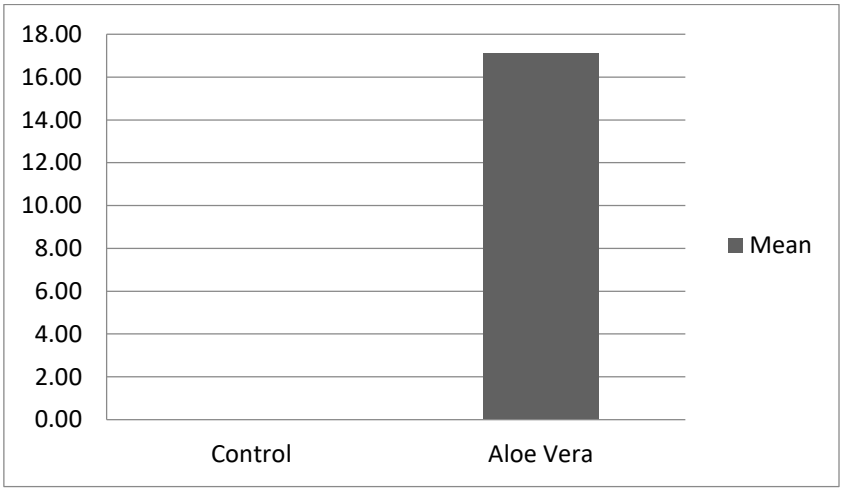
Table 1: Comparison of mean number of Dentine Tubules between 2 groups using Mann Whitney test

Groups	N	Mean	SD	Mean Diff	p-value
Control	15	0.00	0.00	-17.13	<0.001*
Aloe Vera	15	17.13	10.41		

* indicates significant difference at p≤0.05, N- sample size, SD- Standard Deviation



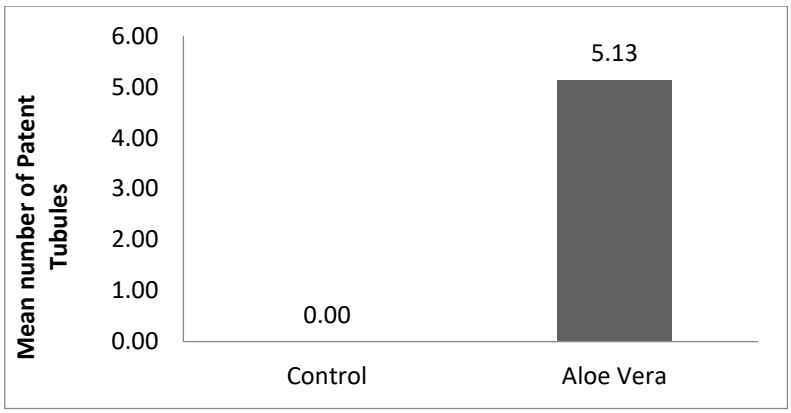
Graph 1: Mean number of Dentine Tubules between 2 groups

The mean number of patent dentine tubules in the *Aloe Vera* group was significantly higher as compared to Control group and the mean difference was statistically significant at p<0.001 (Table 2 and graph 2)

Table 2: Comparison of mean number of patent Dentine Tubules between 2 groups using Mann Whitney test

Groups	N	Mean	SD	Mean Diff	p-value
Control	15	0.00	0.00	-5.13	<0.001*
Aloe Vera	15	5.13	3.34		

* indicates significant difference at p≤0.05, N- sample size, SD- Standard Deviation



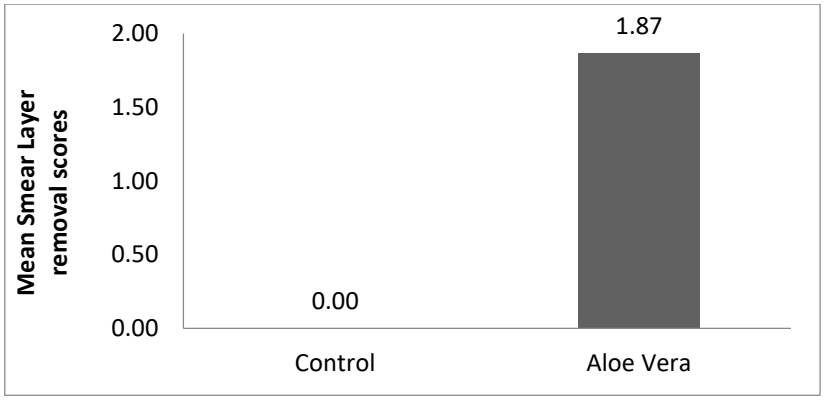
Graph 2: Mean number of Patent Tubules between 2 groups

The mean Smear Layer Removal Scores in the *Aloe Vera* group was significantly higher as compared to Control group and the mean difference was statistically significant at p<0.001. (Table 3 and graph 3).

Table 3: Comparison of mean Smear Layer removal scores between 2 groups using Mann Whitney test

Groups	N	Mean	SD	Mean Diff	p-value
Control	15	0.00	0.00	-1.87	<0.001*
Aloe Vera	15	1.87	0.64		

* indicates significant difference at p≤0.05, N- sample size, SD- Standard Deviation



Graph 3: Mean Smear Layer removal scores between 2 groups

The inference of the present study showed that *Aloe Vera* gel application was significantly more efficient in removing the smear layer, exposing number of patent dentinal tubules than placebo gel respectively.

4. DISCUSSION

It has been demonstrated that the acidic root conditioning solutions eliminate root contaminants and the smear layer after root instrumentation. A stable environment that facilitates the division and migration of cells that takes part in the healing of periodontal wounds and promotes the attachment of connective tissue cells on the surface of the root is created by the demineralization brought on by these agents, which can be linked to the expansion and exposure of the dentinal tubules [16].

The gel used in this trial was passively applied with the help of cotton pellets that had been thoroughly dipped in it for five minutes and reapplied every thirty seconds to ensure a constant delivery of the gel. By chemically removing inorganic material and surface dirt, this process will expose the underlying dentine [17].

Highest smear layer removal and quantity of exposed patent dentinal tubules was seen in aloe vera group than placebo group, suggesting that *Aloe Vera* is an efficient root conditioner.
This was the first study where herbal products have been used as root conditioners. Previous studies have proven to be effective when *Aloe Vera* was used as local drug delivery and topical applicant after flap surgery.

According to a study by Geetha et al. [18], applying *Aloe Vera* gel subgingivally improved periodontal condition, while a study by Anuja et al. [19] discovered that *Aloe Vera* treatment was effective in significantly improving healing. Tetracycline HCL was shown to be more effective than EDTA and doxycycline hydrochloride in a recent study that used SEM analysis to examine the effectiveness of

several root conditioning agents on the elimination of smear layers [9]. The current study employed a similar design, using *Aloe Vera* gel as a root conditioning agent and evaluating the gel's efficacy on the root surface using a histological evaluation method called the SEM, which used better field depth, enhanced resolution, and magnification along the interface.

Although present study showed that *Aloe Vera* is effective when used as root conditioning agent further in vivo studies have to be conducted in future to evaluate the clinical and regenerative capability of *Aloe Vera*.

5. CONCLUSION

In this study *Aloe Vera* gel was found to be efficient root conditioning agent however this in-vitro findings paves the way to evaluate the derived benefits of *Aloe Vera* gel in periodontal tissue healing and regeneration.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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