Clinical Study

Amusha Menezes*
S Ravindra**
Srinath Thakur†
Vidya Dodwad‡

Comparison of a Novel Dual Action Desensitizing Varnish and a Fluoride Varnish - A SEM and In Vivo Study

Abstract

Dental hypersensitivity has affected men since time immemorial and numerous agents are available for treatment of this malady. The exact role of these agents in the management of dentinal hypersensitivity is still unclear and most published studies on dentinal hypersensitivity are of a short duration.

*Assistant Professor,
Dept. of Implantology,
** Professor and Head,
Dept. of Periodontology,
†Professor and Head,
Dept. of Implantology,
‡Professor,
Dept. of Periodontology,
S.D.M. College of Dental Sciences and Hospital, Dharwad.

Address for correspondence:
Dr Amusha Menezes,
Assistant Professor, Dept. of Implantology,
S.D.M. College of Dental Sciences
and Hospital, Davlnagar, Sattur,
Dharwad - 580 009.
E-mail: menezesamusha@hotmail.com
mailamusha@yahoo.co.in

This study was carried out with the aim to:

1. Investigate by sccinning electron microscopy (SEM) the effect of two dentinal varnishes (Fluoritop® and D/Sense2®) on the dentinal tubule orifices, using the 'dentin disc model' and to characterize by X-ray microanalysis, the composition of the deposits, if any, following such an application.
2. Compare the two varnishes in reducing dentinal hypersensitivity, over 6 months.

Introduction

Dentinal hypersensitivity is a problem that has affected men since several years. The causes of this hypersensitivity and effective methods to relieve or prevent it tend to follow traditional routes: First, random treatment, starting with materials of natural origin (roots, plant extracts, animal parts, naturally occurring salts) and then work by the aggressively curious, the intelligent, and the innovative to better understand the physiologic basis of the cause of pain, and the rational methods by which the pain can be treated or avoided[1].

Two approaches for the treatment of dentinal hypersensitivity have been suggested[2], which include partial or complete obturation of dentinal tubules or alteration of pulpal sensory activity at or near the pulpo-dentinal surface. The concept of tubular occlusion as a method of dentin desensitization is a logical conclusion from the hydrodynamic hypothesis. The most commonly used agents in the treatment of dentinal hypersensitivity can be anti-inflammatory agents, protein precipitants, tubule occluding agents, and miscellaneous.

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2. Compare the two varnishes in reducing dentinal hypersensitivity, over 6 months.

Materials and methods

The present study was conducted in the Department of Periodontics, S.D.M. College of Dental Sciences, Dharwad. The study comprised of two parts, *in vitro* and *in vivo*. The *in vitro* SEM analysis and the Energy Dispersive X-ray Analysis were carried out at the National Institute of Oceanography, Goa.

SEM study

Ten freshly extracted caries free unerupted human third molars were used for the *in vitro* study. Extracted teeth were cleaned by soaking for 10 minutes in 3% sodium hypochlorite, then washed with running tap water and stored in distilled water till further use. The procedure followed for obtaining and treating tooth sections was based on the 'dentin disc model' technique described by Gillam, et al.[3]

Procedure for tooth sectioning

The teeth were cut transversely with a diamond sectioning saw microtome under running water to sections of 1 mm thickness. Each tooth yielded 2-3 dentin sections; therefore 10 teeth gave a total of 26 discs. The discs were ultrasonicated in distilled water for 30 seconds, etched with 6% citric acid for 2 minutes to remove the smear layer, followed by rinsing in distilled water. Each disc was marked on either side with small notches to assist in identification and orientation of both halves after splitting the section. Discs were then split into two halves using a dental chisel. One half was used for Fluoritop® and the other half for D/Sense2®. This disc halving procedure allowed strict comparison to be made between the two specimens.

Treatment of discs

Sections were initially soaked in saliva supernatant (0.877 g sodium chloride, 1.491 g potassium chloride, 0.735 g calcium chloride, 1.260 g sodium bicarbonate and 3.5 g mucin dissolved in distilled water and titrated to pH 6 to obtain a final volume of 1000 ml). Fluoritop® varnish application was carried out for one-half (n = 24) of all the specimens and the other half (n = 24) were treated with D/Sense2® varnish. Notched pairs were reoriented and rinsed in distilled water for 10 seconds and then processed for SEM. Four separate discs were treated with only distilled water and these served as controls for comparison of surface changes.

SEM preparation

Discs were then prepared for SEM. From parallel specimens for each agent, a treated disc half was further fractured into two quarters using dental pliers without touching the treated disc surface, allowed to dry in a dessicator for 2 days and examined under magnification of 1000X and 2500X using a scanning electron microscope, to determine evidence of tubule penetration of the two different applications.

SEM examination

Each SEM micrograph was ranked according to the scale described. In a blind evaluation, a single investigator ranked the micrographs. Each micrograph was studied for semiquantitative assessment of number of dentinal tubules occluded in that particular field and the score was recorded in the proforma designed.

Rank	Number of dentinal tubules occluded in the micrograph
1	None
2	Upto 1/3 of the tubules
3	1/3-2/3 of the tubules
4	>2/3 of the tubules

Four arbitrary fields were chosen for each half of the disc and the mean dentinal tubular occlusion was calculated.

X-ray microanalysis

X-ray microanalysis was done on the samples, to characterize the nature of deposits, if any. Samples were analyzed using the energy dispersive compo-sitional analyzer.

In vivo study

Thirty male and female subjects ranging from 18 to 60 years and in good general health who exhibited cervical dentinal hypersensitivity, as demonstrated by pain elicited by the two test stimuli, in relation to atleast two teeth, were selected for the study. Pairs of teeth on opposing sides of the mouth, with comparable sensitivity ratings within the subject

were randomly assigned one to the Fluoritop® group (Group F) and the other to the D/Sense2® group (Group D). The teeth also showed evidence of buccal cervical abrasion, erosion or recession. The two test stimuli used for the screening and selection procedure were as follows:

- Sensitive teeth identified with a straight probe used cervically on each tooth anterior to the second molar
- Ten minutes later, the tooth response to cold air was assessed, using a standard dental air syringe.

Teeth were excluded from consideration if there was excessive gingival inflammation or bleeding on probing from the adjacent gingiva, if they had a dental pathology, which caused pain similar to cervical dentinal hypersensitivity (teeth with caries or enamel cracks), if they were partial denture abutments or had mobility greater than Grade I, had cervical restorations, crowns or bridgework.

Tests for recording cervical dentinal hypersensitivity

Tactile method

- **Probe score**: The hand held scratch device consists of a tension gauge and a sharp explorer like probe that could be passed easily across a sensitive tooth. It has an indicator that is displaced by the arm of the explorer tip that records the force of displacement in centiNewtons (cN). The scratch process was reported with successively greater force in increments of 5 cN until pain was felt. That force was considered the pain threshold for the teeth under test and was noted in cN. The maximum force applied was 80 cN. If following the initial visit the subject did not perceive any discomfort at that setting (i.e., 80 cN) a score of 80 was recorded.
- **Probe intensity score**: The subject was also asked to rate the perception of the sensitivity experienced during scratch process by providing a score of 0 -10 (where 0 = no pain and 10 = excruciating pain) based on a numerical rating visual analogue scale (NRS) described by Gillam, et al.

(Thermal/evaporative method)

Cold air score

Exactly 10 minutes after the scratch response, response to a 1 second application of cold air from a dental unit syringe (at 20°C ± 3°C at 60-65 psi).

The subject was again asked to rate the perception of sensitivity experienced during this test by providing a score of 0-10 (where 0 = no pain and 10 = excruciating pain).

Subjective reporting of pain at baseline (overall sensitivity)

Subjects were also asked to rate their perception to hot/cold food and drink, air, tooth brushing and to sweet and sour food by providing a score of 0-10 (0 = no pain and 10 = excruciating pain).

Following testing, the subject's hypersensitivity was graded as 'mild' or 'severe' depending on the average 'cold air score' at baseline (day 0). An average of ≤5 was graded 'mild' while >5 was graded 'severe'. The 'mild' and 'severe' hypersensitive teeth were then randomly allocated to Group F or Group D.

Application of varnish

Fluoritop varnish

The selected teeth were dried, and recommended quantity of varnish (0.2-0.5 ml) was painted on the tooth surface with the help of the applicator brush. The varnish was then allowed to dry for 5 minutes. This application was carried out once a week for 2 weeks.

D/Sense2

The selected teeth were dried. Three drops of the kit's Step 1 Liquid which contains water potassium phosphate, potassium carbonate and sodium methyl paraben was dispensed. Step 1 Liquid was liberally brushed onto the dentin surface for 10 seconds. Three drops of the kit's Step 2 Liquid, which contains water, sodium benzoate and the inorganic salts calcium chloride and strontium chloride, was dispensed into another Bond-Logic Well, taking care not to mix the Step 1 and Step 2 Liquids in the Bond-Logic Wells together. Using a clean yellow Benda Brush from the kit, Step 2 Liquid was applied onto the tooth surface, and agitated for 10 seconds. Both the liquids reacted instantly and crystallized to close the tubules.

Subjects were instructed not to brush, rinse or chew anything for 4 hours after application of the varnishes. Subjects were advised to brush their teeth using a soft

toothbrush and non-fluoridated toothpaste twice daily.

Subjects were reassessed at 1 week, 1 month, 3 months and 6 months and all tests described above were recorded again.

Statistical analysis

Results of the study were subjected to statistical analysis, by applying the following tests. Between and within groups data for all treatment outcome measures were analyzed by paired and unpaired 't' test. For analysis of SEM and tactile pressure, since measurements were made on ranks and scores (cN), non-parametric tests were used for analysis. For all tests of significance, a p value of either 5% ($p < 0.05$), 1% ($p < 0.01$) or 0.1% ($p < 0.001$) was taken.

Results

In vitro

The 26 dentin discs yielded from 10 freshly extracted teeth were split into 52 halves and divided into three groups:

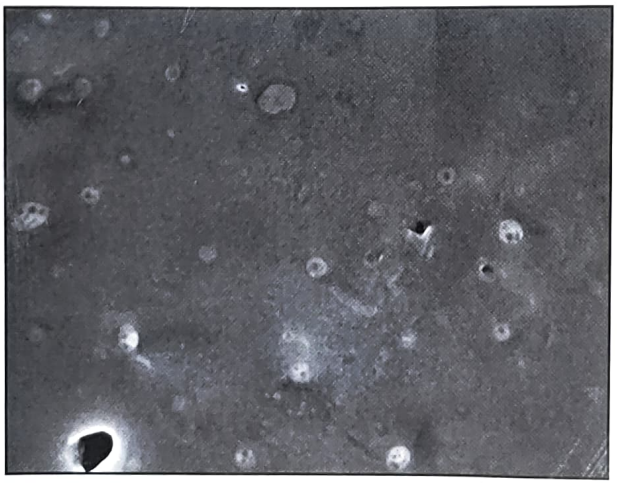
- Group F (n = 24)
- Group D (n = 24)
- Control (n = 4).

All specimens from the Group F and Group D were subjected to treatment with Fluoritop® varnish and D/Sense2® varnish, respectively. After treatment the specimens were subjected to SEM analysis. Micrographs were studied from four randomly selected fields on both test and control sections in the central portion of the disc.

The active ingredients of Fluoritop®, produced a granular precipitate that occluded a high proportion of the tubules (see micrograph 1A). After fracturing, the sections, it was noted that, this precipitate penetrated the tubules to some depth (see micrograph 1B and 1C). On the other hand, D/Sense2® also produced variable coverage of the dentin surface, with a crystalline precipitate. (see micrograph 2 A). After fracturing, the sections, it was noted that, this crystalline precipitate showed minimal penetration of the tubules. (see micrograph 2 B and 2 C). The untreated control discs too, showed negligible, few stray deposits, which did not produce any visible effect on the dentinal tubule orifices i.e., there was no dentinal tubular occlusion seen (see micrograph 3).

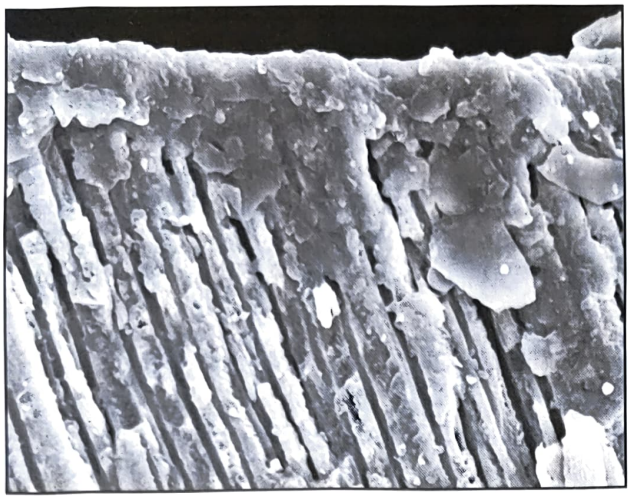
The quantitative assessment showed that the level of coverage and occlusion of the tubules varied minimally between the products and no significant difference was found. (Table 1 and Fig. 1).

To verify the elemental nature of these, the deposits on these specimens were subjected to energy dispersive X-ray analysis (EDXA). The results of X-ray analysis of Fluoritop® treated discs showed peaks of fluoride, calcium, carbon and oxygen and minute traces of nitrogen, sodium, magnesium, aluminium, silica and phosphorous (see spectral graph I). Conversely, the results of X-ray analysis of D/Sense2® treated discs showed peaks of, calcium, carbon, oxygen phosphorous and strontium and minute traces of nitrogen, sodium, magnesium, aluminium and silica (see spectral graph II). However, the control specimens, showed peaks of carbon, nitrogen, oxygen and calcium and traces of sodium, magnesium, aluminium and silica (see spectral

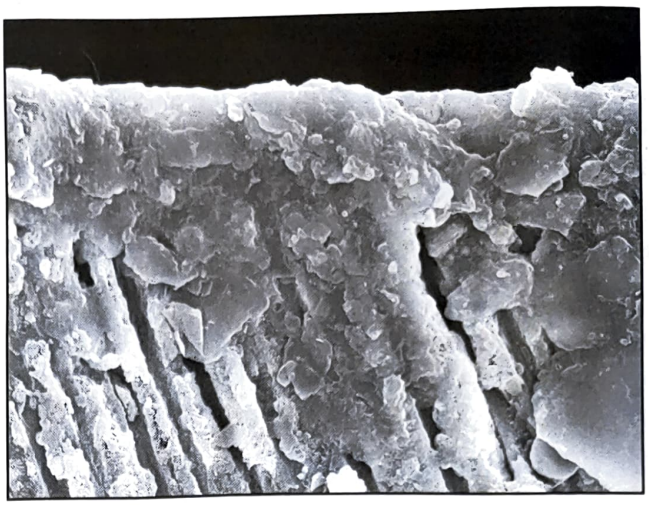


Micrograph 1A. Surface deposition of Fluoritop varnish treated discs.

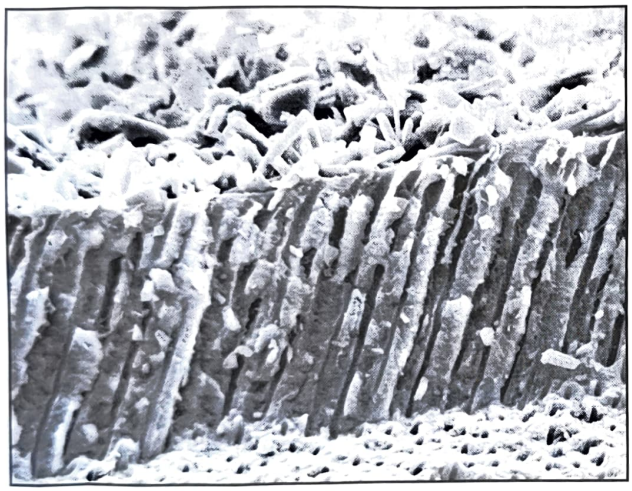
Micrograph 2A. Surface deposition of D/Sense2 varnish treated discs × 2500.



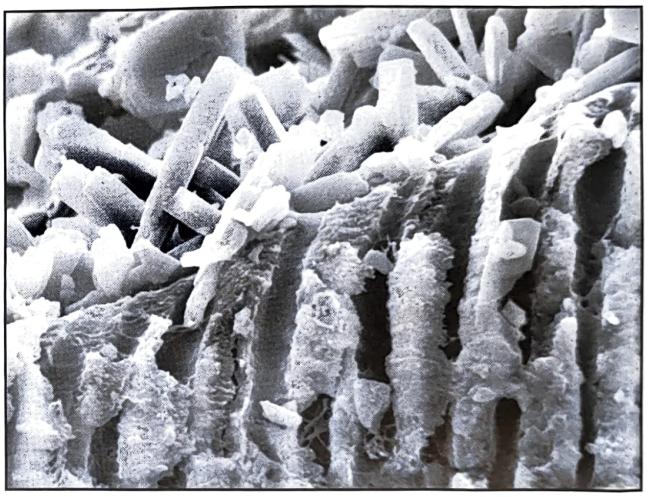
Micrograph 1B. Section of a fractured dentin disc treated with Fluoritop varnish × 1000.



Micrograph 1C. Section of a fractured dentin disc treated with Fluoritop varnish × 2500.

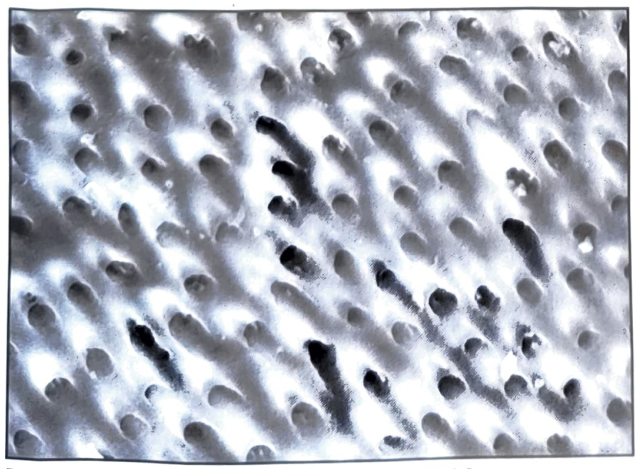


Micrograph 2B. Section of a fractured dentin disc treated with D/Sense2 varnish × 1000.



Micrograph 2B. Section of a fractured dentin disc treated with D/Sense2 varnish × 2500.

graph III). This confirmed that the deposits in the two test groups were from the respective active agents. In the control however, the deposits might have been part of the tooth structure.



Micrograph 3A. Untreated discs × 2500.

In vivo

Both groups exhibited significant reduction in mean sensitivity over the 6 months period from the baseline (day 0). Within Group F and D, it was seen that, the efficacy of both the agents reduced after 1 month and the reduction was more in Group D compared to Group F. (see Table 2 and Fig. 2).

Discussion

In this *in vitro* study, the 'dentin disc model'[3,5] was used. This model was used in several earlier studies by

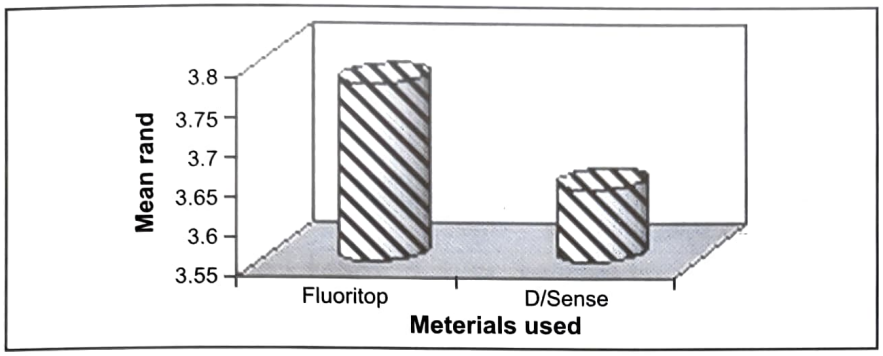


Figure 1. Showing comparison of SEM evaluation the dentin discs treated with Fluoritop and D/Sense2

Table 2. Comparison of overall sensitivity scores (0-10 intensity scale) between Group F and Group D at baseline/1 week/1 month/ 3 months and 6 months

Period	Mean D	Mean F	SD D	SD F	t value	p value	Signif
Baseline	7.8333	7.9333	1.0854	1.1427	0.3475	>0.05	NS
1 week	0.0000	0.0000	0.0000	0.0000	0.0000	>0.05	NS
1 month	3.4333	0.4333	1.5241	0.6789	9.8481	<0.01	HS
3 months	5.9667	5.9667	0.9643	1.1885	0.0000	>0.05	NS
6 months	6.9000	6.6000	0.8847	0.8550	1.3355	>0.05	NS

Group F (n = 30), Group D (n = 30)

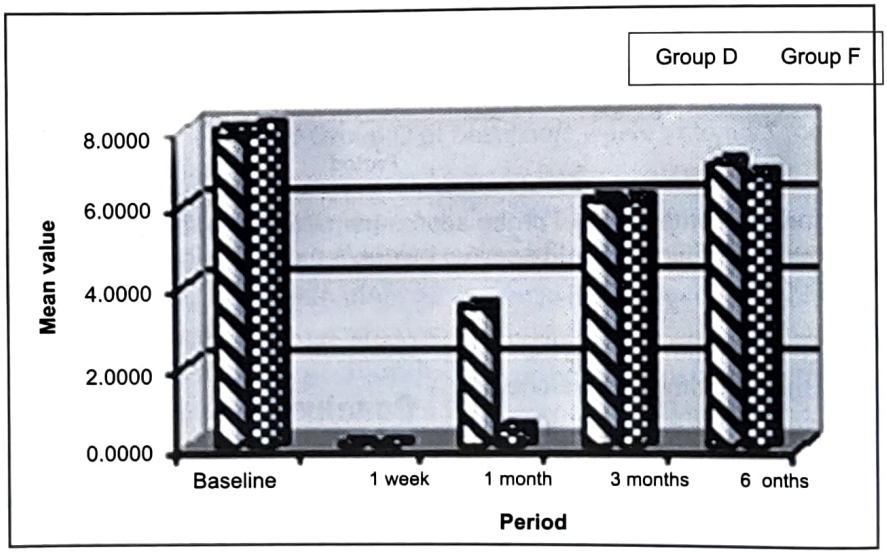


Figure 2. Comparison of overall sensitivity scores (0-10 intensity scale) between Group F and group D at baseline/1 week/1month/ 3 months and 6 months.

Greenhill and Pashley[6] and Gillam, et al.[3] and is considered to represent a close approximation to the *in vivo* situation. The teeth specimens were treated with the respective varnishes in accordance with the method described by Gillam, et al[3] and Srinivas, et al.[7]

This study showed that the dentin discs treated with fluoride varnish, produced obliteration of tubule orifices, by an amorphous granular precipitate, suggesting that the components of the varnish have tubule occluding properties. This was in correlation with several investigators who have also found a granular precipitation after studying the scanning electron micrographs of dentin treated with 0.2% sodium fluoride (Ehrlich et al)[8].

Similarly dentin discs treated with D/Sense2® varnish, produced obliteration of tubule orifices, by crystalline precipitate. Since D/Sense2â also has potassium ions it could imply that in addition to tubule occlusion, there could be a different mechanism of action such as may be, an alteration of neural transmission at the dentino-pulpal junction, and which could not be detected by this *in vitro* model.

In this study the control specimens showed a few stray negligible deposits. The EXDA of the deposits to elicit its composition in the Fluoritop treated discs showed peaks of fluoride, carbon and oxygen and minute traces of nitrogen, sodium, magnesium, aluminium, silica and phosphorous, indicating that the active agent fluoride was present in the precipitate that occluded the tubules. However, D/Sense2® treated discs showed peaks of, calcium, carbon, oxygen phosphorous and

strontium and minute traces of nitrogen, sodium, magnesium, aluminium and silica. Potassium was not detectable. According to Maccaroni et al.[11], the soluble potassium chloride salt infuses into the tubules and a 3-micron crystalline complex seals it (calcium phosphate/calcium carbonate/strontium phosphate/strontium carbonate). In the control specimens, peaks of carbon, nitrogen, oxygen, calcium and traces of sodium, magnesium, aluminium, silica and phosphorous were detected, these deposits could have been tooth debris, though the source of these deposits is unknown.

The clinical study was a split mouth, controlled comparative longitudinal evaluation of two parallel groups over a 6-month period. Thirty subjects, with a chief complaint of dentinal hypersensitivity and having atleast two bilateral teeth, which were sensitive, were selected for the study.

The results from the clinical study showed that fluoride varnish was an effective desensitizing agent. This in correlation with that of Srinivas, et al.[7], Ehrlich, et al.[8], Mc Fall, et al. and Kerns, et al[13]. We found that the efficacy of both the varnishes reduced after 1 month following application and the reduction in efficacy was more for the D/Sense2® group compared to the Fluoritop® group.

In this study we did not have a placebo because according to Gillam[4], in a split mouth design study it is possible to have a superiority trial, which involves the evaluation of a therapy to show whether it is efficacious and superior to a standard accepted therapy.

Table 3. Comparison of probe scores (centi Newtons) between Group F and Group D at baseline/1 week/1 month/3 months and 6 months

Monn-whitney U test

Period	Mean D	Mean F	SDD	SDF	Z value	p value	Signif
Baseline	44.0000	43.5000	5.9306	0.0000	–0.3992	>0.05	NS
1 week	80.0000	80.0000	0.0000	0.0000	0.0000	>0.05	NS
1 month	79.0000	79.0000	2.8342	2.0000	–0.3548	>0.05	NS
3 months	60.0000	66.6670	6.3000	7.5000	–3.3265	<0.01	HS
6 months	46.8333	58.7667	6.4000	7.8000	–4.9306	<0.01	NS

Group F (n = 30), Group D (n = 30)

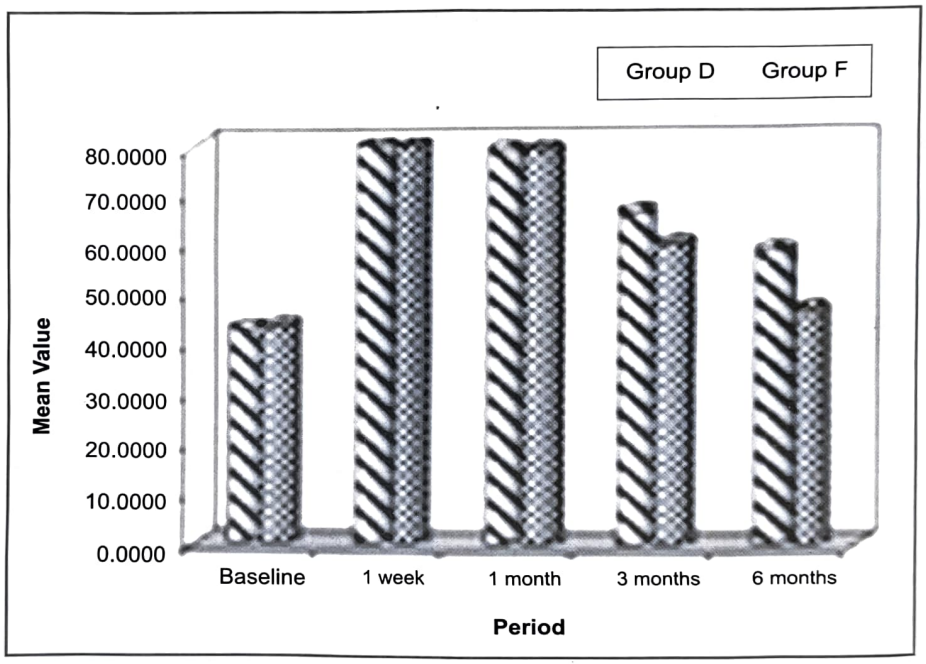


Figure 3. Comparison of probe scores (centiNewtons) between Group F and Group D at baseline/1 week/1 month/3 months and 6 months.

The major limitation of the study was the inability to simulate the oral conditions *in vitro*. The highly variable nature and composition of saliva constituents between individuals and the simulation of the same *in vitro* is difficult. Hence correlation of the results obtained *in vitro* and *in vivo* should be done with caution.

Conclusion

From the present study, the following conclusions can be drawn:

- By using the dentin disc model described by Gillam[3], the results from the present *in vitro* study

Table 4. Comparison of probe intensity scores (0 –10 intensity scale) between Group F and Group D at baseline/1 week/1 month/3 months and 6 months

Unpaired 't' test

Period	Mean D	Mean F	SD D	SD F	t value	p alue	Signif
Baseline	6.6667	6.7333	1.1547	1.1427	–0.2248	>0.05	NS
1 week	0.3333	0.0000	0.1826	0.0000	1.0000	>0.05	NS
2 months	0.9000	0.3000	0.9948	0.5350	2.9094	<0.001	HS
3 months	5.4667	1.2667	1.2243	0.9072	15.0971	<0.001	HS
6 months	6.2333	2.2333	1.2229	0.9714	14.0284	<0.001	HS

Group F (n = 30), Group D (n = 30)

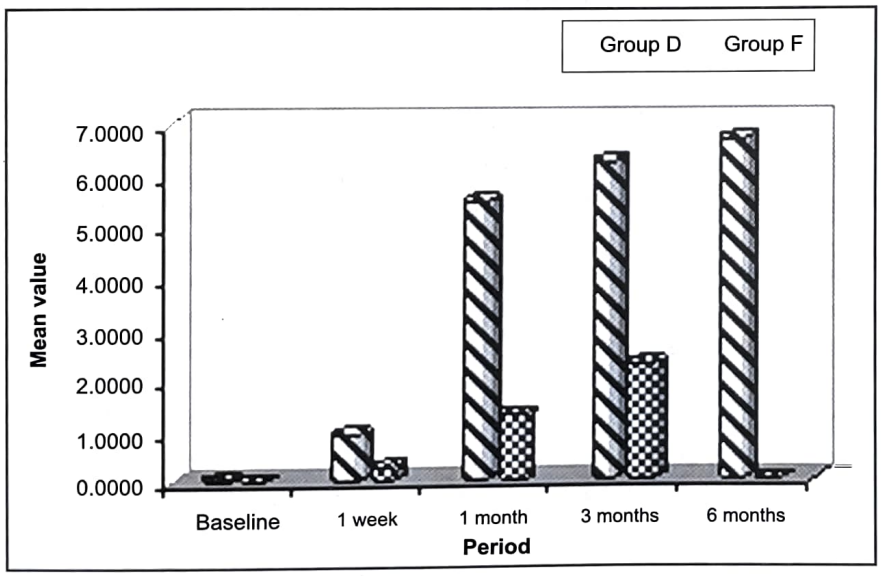


Figure 4. Comparison of probe intensity scores (0-10 intensity scale) between Group F and Group D at baseline/1 week/1 month/3 months and 6 months.

indicate that both the fluoride varnish as well as the varnish containing a combination of potassium phosphate, potassium carbonate, calcium chloride and strontium chloride brought about tubule occlusion.

In the *in vivo* situation the varnish may employ mechanisms that do not entirely rely upon tubule occlusion for treatment for dentinal hypersensitivity.

- There was a general decrease in dentinal hypersensitivity levels in both groups upto the 1-month period as demonstrated by all four methods of assessment. However from one month to six months there was again a gradual increase in hypersensitivity for both the groups. However the increase in sensitivity was more in the D/Sense2® group compared to the Fluoritop® group.

Fututre directions

In the future it would be interesting to carry out studies monitoring the effects of these agents for treating hypersensitivity, and for determining the number of applications and amount of drug to be used. By determining the same, it may be possible to optimize the effects of this treatment modality.

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