Scanning electron microscopic evaluation of the desensitizing effect of propolis in the dentine disc model: An in-vitro study

Aamir Rashid Purra, Mubashir Mushtaq

ABSTRACT

Aims: To assess the efficacy of Propolis as a desensitizer to occlude open dentine tubules in the dentine disc model and the degree of retention of the surface deposit upon rotation with saliva supernatant for 6 hours. Materials and methods: Caries free surgically extracted maxillary and mandibular molars were used for this study. One half of the specimen served as the control and the other half was treated with test agent. The control halves were placed in a desiccators for 1-2 days to dry and the test halves were treated with a single application of propolis using a fine brush. The study specimens were observed under the scanning electron microscope at a magnification of 3000X. The photomicrographs, one from each test and control surface, were evaluated by two independent observers who were not aware of the test solution and the scores were ranked. The consistency of the ranking procedure was tested by Cohen’s kappa (K). A value of K>0.75 was considered good examiner consistency. Mean scores were calculated for each group and statistically evaluated. Results: Comparison of the mean scores for control and Propolis group revealed significant difference in the tubule occluding effects between the two groups. Conclusion: It was concluded that Propolis occluded substantial number of tubules and there was a uniform stable coverage across dentin disc surface.

Key words: Dentinal hypersensitivity; Dentin disc model; Propolis.

Introduction

Dentinal hypersensitivity is a common clinical condition which affects between 8 to 35% of the population. There is a consensus that sensitivity arises from fluid movement within the tubules stimulating mechanoreceptors on the pulpal nerves as stated by hydrodynamic dynamic theory of Brannstrom. No completely reliable treatment has been put forward for dentinal hypersensitivity. An agent can be of benefit in one case and fail in another. Due to the fact that dentine sensitivity is a very prevalent condition, a variety of symptomatic treatments exist. The requirements for an ideal treatment for dentin sensitivity were listed by Grossmann in 1935. Search for an ideal agent still continues.

Natural products have been used for thousands of years in folk medicine for several purposes. Among them, propolis a bee product has attracted increased interest due to its harmless nature and innumerable biological activities. Propolis is a resinous yellow brown to dark brown substance collected by honey bees from sprouts, exudates of trees and other parts of plants and modified in the beehives by addition of salivated secretions and wax. It is used by bees for protection, to repair openings and damages in hives, to construct aseptic places and secretions and wax. It is used by bees for protection, to repair openings and damages in hives, to construct aseptic places and to embalm killed invaders. Chemically, propolis of different parts of the world is constituted by 50-60% of resins, 30-40% of waxes, 5-10% of essential oils, 5% pollen, besides microelements like aluminum and calcium. So far, more than three hundred organic compounds of different groups mainly phenolic, such as: flavonoids, stilbenes, phenolic acids and its esters have been identified from propolis. Many biological activities have been reported for propolis, such as antimicrobial, antimalarial, antiviral, anti-inflammatory, antitumor, antioxidant, anaesthetic and free radical scavenging action.

A pioneering pilot study was undertaken by Khalid Almas et al on the effect of propolis on dentinal hypersensitivity in vivo for a study period of four weeks. It was concluded that propolis had a positive effect in control of hypersensitivity. The authors explained the effect due to blockade of dentinal tubules in light of their SEM observation. The authors found that application of propolis for 1 and 2 minutes caused blockage of dentinal tubules. Propolis caused a stable coating on the dentine surface and water irrigation did not have effect on the coating of the agent. According to these authors, it may be presumed that tubule occlusion produced by the propolis may last to permit natural physiological reaction to occlude the tubules and maintain desensitization.

Extensive literature review showed that this was the only study that assessed the effect of propolis on dentine tubule blockage and more investigations are required. No study till date has been done to see the effect of propolis on smear layer removal and opened dentinal tubules which clinically mimic the situation of dentinal hypersensitivity.

In the present study, the effect of propolis have been assessed on the exposed dentin surface and compared with a control. Scanning electron microscopy (SEM) was used to provide a qualitative indication of the degree of tubule occlusion on the exposed dentine surface in the dentine disc model.

Materials and Methods

Caries free surgically extracted maxillary and mandibular molars were used for this study. The teeth were fixed in 3% Glutaraldehyde in 0.1m sodium cacodylate buffer solution (pH 7.4) for 1 week. After all organic matter had been cleaned from the fixed tooth surface; the teeth were stored in 0.1 m sodium cacodylate buffer solution and subsequently sectioned mesiodistally into discs approximately 1mm thick using a diamond
The photomicrographs, one from each test and control subular material of probable odontoblastic origin.

Results

The photomicrographs, one from each test and control surface, were evaluated by two independent observers who were not aware of the test solution and the scores were ranked according to following criteria:

Score 1: All tubules open.
Score 2: <25% tubules covered.
Score 3: >25% but <50% tubules covered.
Score 4: >50% but <75% tubules covered.
Score 5: Very heavy coverage, few or no tubules visible.

The consistency of the ranking procedure was tested by Cohen’s kappa (K). A value of K>0.75 was considered good examiner consistency. Mean scores were calculated for each group.

Control group: In cut dentine discs after etching in 6% citric acid for 2 min, the smear layer was removed and the tubule openings were clearly visible (Figure 1). Towards the center of the discs the tubules demonstrated a more or less circular profile. The control showed all ten samples had score of 1 with mean score 1 i.e., all tubules were open (Table 1).

Propolis group: After a single 5 min application of propolis there was a noticeable deposition of amorphous products across the disc surface with most of the tubules being occluded (Figure 2).

Three samples had a score of five and rest seven samples had a score of four. The mean score was 4.2 i.e., greater than 75% of tubules being blocked (Table 1).

Comparison of the mean scores for control and Propolis group revealed significant difference in the tubule occluding effects between the two groups.

Discussion

The purpose of this invitro study was to evaluate the potential tubule occluding properties of propolis using a dentine disc model. The dentins disc model has been used in several earlier studies considered to represent a close approximation to the in-vivo situation. It is a useful model for the in-vitro examination of the physical barrier aspect of the complex phenomenon of dentinal hypersensitivity as it is readily available.
and can be used with a wide range of exposures. However, experimental procedures should be rigid and results interpreted with care. The method described in this study differs from others in standardization of a control and in the strict protocol employed regarding areas to be viewed.\(^{13}\)

In the present study, the dentine disc model was employed to examine the tubule occluding properties of these agents and to relate the results, where possible, to the clinical situation. Most studies on tubule occlusion have focused on coronal dentine where important variables such as dentine surface area, thickness and surface characteristics can be controlled. The validity of data collected in vitro, however, is open to criticism. The hydraulic conductance of radicular dentine has been observed to be much lower than that of coronal dentine. There is a good correlation between tubule density and diameter and the measured hydraulic conductance. If the design of the experiment would allow a curved treatment surface, it might be preferable to use cervical dentine blocks for the screening of potential desensitizing agents.\(^{14}\) For the purpose of the present study, where standardized comparison of the different treatment procedure was required, a dentine disc model prepared from the coronal tooth structure appeared appropriate, given the variation in tubule density and diameter.\(^{14}\)

The tubules in the middle of the disc, above the pulp horns will have a wider diameter than those at the periphery to the disc surface but they are oriented perpendicular to the disc surface, and therefore offer the best test surface.\(^{15}\)

Propolis caused substantial tubule occlusion. Mean complete occlusion was greater than 75%. The dentine surface was covered with granular layer and all tubules partly or completely reduced in diameter. The change if extrapolated to clinical effect would suggest that relief from dentine hypersensitivity would be immediate. Evidence comes from the pioneer study of Almas et al found immediate relief in majority of patient on application of Propolis.\(^{8}\)

As with all in-office treatments, the persistence of tubule occlusion is the important parameter.\(^{14}\) The deposits formed by propolis were little influenced by rotation in artificial saliva. This would attest to the apparent strong affinity of deposits for dentine and possible could be the cause of lasting relief from dentine hypersensitivity in the above study. Interpreting changes in in-vitro is difficult and extrapolation into the clinical situation must be tempered with caution. The results of the present study failed to show any observable changes mediated by the active ingredients contained in the test agents. This could relate to the fact that different mechanisms of action, such as alteration of neural transmission, at dentino-pulpal junction or some form of protein precipitation within dentinal tubules or long term formation of reparative dentine may operate in the desensitizing process. Such mechanisms, however, would not be stimulated in the dentine disc model. Moreover the relevance of the argument in present case is substantiated by the fact that propolis does show anaesthetic effect as well has shown to induce formation of reparative dentine in rats.\(^{15}\)

**Conclusion**

To conclude, the results of the present in-vitro study demonstrated that propolis has substantial potential for occluding dentinal tubules. Further quantitative and functional studies, however, are required to determine the effects of propolis on dentine permeability in vivo, the nature of deposits so formed as well as clinical studies to evaluate its potential in reducing dentine hypersensitivity over time in comparison to other currently used desensitizing agents.

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**References**


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