In vitro evaluation of the effect of natural orange juices on dentin morphology

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Abstract: The patient's diet has been considered an important etiological factor of dentin hypersensitivity. The frequent ingestion of acidic substances can promote the loss of dental structure or remove the smear layer. The purpose of this study was to evaluate the degree of smear layer removal and dentinal tubules exposure by different natural orange juices. Extracted human teeth were submitted to manual scaling in order to develop the smear layer. Seventy dentin samples were obtained and distributed into the following groups: Control, lime orange, lime, valência orange, navel orange, mandarin, and tangerine. Each group included 2 methods of application: Topical and topical + friction. After preparation for SEM analysis, photomicrographs were assessed by a blind calibrated examiner using an index system. The Kruskal-Wallis test indicated a significant influence of the orange juices on smear layer removal. Significant difference was observed between navel orange, valência orange, mandarin and the control group ($p < 0.05$). These orange juices resulted in greater removal of the smear layer and greater opening of dentinal tubules. The comparison between the application methods for each group using the Mann-Whitney test showed that friction increased smear layer removal significantly only for lime orange and lime. The data suggest that certain natural orange juices are more effective in terms of smear layer removal and dentinal tubules exposure than others.

Descriptors: Dentin sensitivity; Diet; Smear layer.

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Introduction

Dentin hypersensitivity (DH) can be described as a common painful condition arising from exposed dentin in response to chemical, thermal, tactile, or osmotic stimuli that cannot be explained as resulting from any other form of dental defect or pathology.[1]

Based on the hydrodynamic theory for stimulus transmission across dentin,[2] it must be assumed that patients exhibiting DH have tubules open at the dentin surface through to the pulp.[3] However, the factors that expose dentin and open dentinal tubules are not clearly established. Most attention has been directed towards treatment and little emphasis has been placed on the knowledge of etiological factors, which are essential to prevention, to treatment and to avoid recurrences of this condition.

DH appears to have a multifactorial etiology,[1,4,5] but previous studies have demonstrated that one of the most important factors is the patient's diet.[3,6-9] Evidence in *in vitro* studies indicate that acidic substances can cause the loss of dental structure (erosion) or remove the smear layer and expose dentinal tubules.[3,6,7,10] In addition, Clark *et al.*[11] (1990) observed a negative association between the frequency of ingestion of specific acidic foods and beverages and the persistence of DH. On the other hand, the effect of toothbrushing immediately after the exposure of dentin to acidic substances is controversial. Some studies state that brushing in the presence of dietary acids can enhance smear layer removal and tubule opening,[6,8] while others suggest that toothbrushing creates a new fine and thin smear layer and reduces dentin permeability.[9]

Dietary counseling regarding the quantity and frequency of acid intake should be an essential part of DH management.[5,7,11] It is certain that any treatment undertaken which does not take into account the control of such etiological factors will only be partially successful and of short duration.[1,5] So, the aim of this *in vitro* study was to observe, using a scanning electron microscope (SEM), the effect of different types of natural orange juices on smear layer removal and dentinal tubules exposure. Secondly, to evaluate the effect of toothbrushing subsequent to orange juice application.

Material and Methods

Twenty-five human teeth recently-extracted for periodontal reasons were used. Although there was no criterion regarding tooth type, the teeth should not present any caries and the root surface should be intact. The teeth were obtained after informed consent by the patients was obtained and after the study protocol was approved by the Ethics Committee, School of Dentistry at Araraquara, São Paulo State University (UNESP).

The dentin specimens were prepared as described by Corrêa *et al.*[7] (2004). High-speed diamond-coated burs (n. 3202 - KG Sorensen, Barueri, SP, Brazil) were used to remove the cementum from the cervical portion of the roots. Subsequently, to form the smear layer, the teeth were instrumented with forty shaving strokes in each surface using Gracey's curettes 5-6 (Hu-Friedy, Chicago, IL, USA) by the same operator. These roots were then reduced with a diamond disk (KG Sorensen, Barueri, SP, Brazil) to obtain three dentin samples of each tooth (3 x 3 x 1 mm) that were stored in a container with saline solution (Merck, Darmstadt, Germany) to avoid dehydration. A total of 70 samples were used and randomly divided into negative control (distilled water) and six experimental groups of natural orange juices: Lime orange, lime, valencia orange, navel orange, mandarin and tangerine. The fruit juices were prepared by the investigators immediately before the experiment, and the pH of each was determined at that time by a single electrode pH meter (pH Meter, UB-10, Denver Instrument, Arvada, CO, USA). The juices were not buffered, since pH may be a factor in smear layer removal and, consequently, may influence DH.

Each experimental group and the control group consisted of 10 samples, subdivided into 2 groups of 5 samples each. Fruit juice was applied to the samples by one of the following methods[7,12]:

- Topical: Samples were immersed in the juice for 5 minutes and washed with a stream of tap water for 15 seconds.
- Topical with friction: Samples were immersed in the juice for 5 minutes, manually brushed by the same operator with a soft toothbrush (Colgate, São Bernardo do Campo, SP, Brazil) for 30 sec-

onds and washed with a stream of tap water for 15 seconds.

For SEM analysis (Jeol T330 A, Jeol Ltd., Peabody, MA, USA), the samples were dehydrated in ethanol solutions (Merck, Darmstadt, Germany) of increasing concentrations (30, 50, 70, 80, 95 and 100%), dried overnight in a dehydration jar (Corning, São Paulo, SP, Brazil), mounted on metallic stubs (Senai, Araraquara, SP, Brazil) and sputter-coated with gold. Two photomicrographs were obtained from the center of each sample, with magnifications of 750 and 1,500 X, that were subsequently assessed by an examiner previously calibrated and blind to the experimental groups using the index of smear layer removal[7,12]:

- Score 1 - Complete removal of the smear layer, dentinal tubules open.
- Score 2 - Partial removal of the smear layer, dentinal tubules partially open.
- Score 3 - Smear layer present on the dentin surface, indication of opening of dentinal tubules.
- Score 4 - Smear layer present on the dentin surface, total obliteration of dentinal tubules.

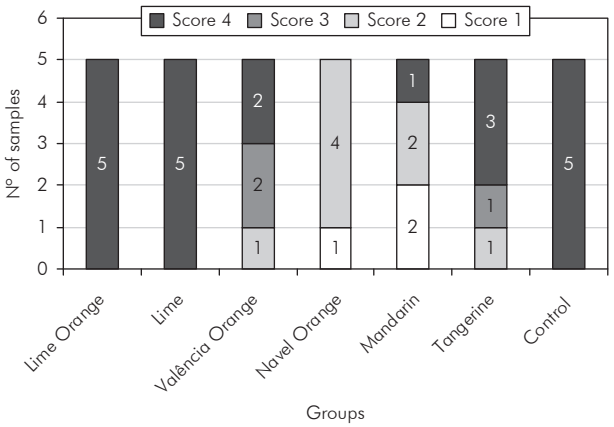
The examiner accomplished three consecutive readings, with intervals of one week, for each photomicrograph. The predominant score of the three readings was considered representative of the respective sample. The Kappa test was applied twice after scoring subsets of samples to check the calibration of the examiner and the reproducibility of his readings, as a means to assure the consistency of the evaluation. The values obtained with the Kappa tests were 0.7 and 0.9.

The data were obtained using an index representing a scoring system, so non-parametric analyses were applied. Considering each method of application separately, evaluation of the treatment factor influence on the scores attributed to the samples was performed by a non-parametric analysis of variance (Kruskal-Wallis), followed by the Dunn test for multiple comparisons. On the other hand, the Mann-Whitney test was applied to compare results between the two methods of application for each orange juice tested. A 5% confidence level was used and the calculations were performed with the software Statistica, version 5.1 (Statsoft Inc., Tulsa, OK, USA).

Results

Graph 1 represents the frequency distribution of scores within each group treated by topical application. In this case, the Kruskal-Wallis test indicated a statistically difference between substances ($p = 0.0009$), with *post-hoc* paired comparisons demonstrating that only navel orange (Figure 1) was significantly different from the control ($p < 0.05$) (Figure 2). This group presented the lowest average rank, which indicates greater removal of the smear layer and greater opening of the dentinal tubules.

The results for the active (topical + friction) application of the tested juices were different from those of the topical application (Graph 2). The Kruskal-Wallis test indicated a significant difference among groups ($p = 0.0003$), and the *post-hoc* paired



Graph 1 - Frequency distribution of scores for the samples of each group that received topical application.

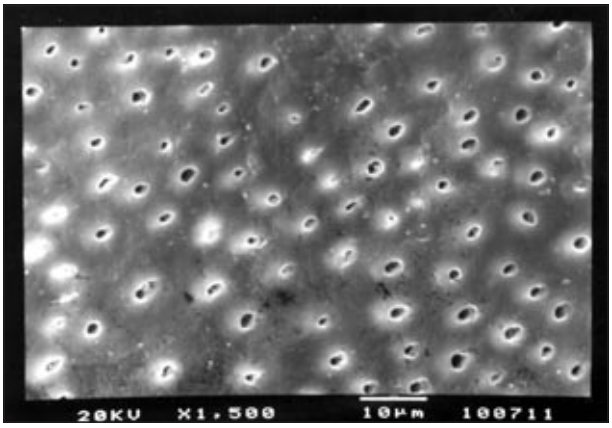


Figure 1 - Photomicrograph of sample treated by topical application of navel orange.

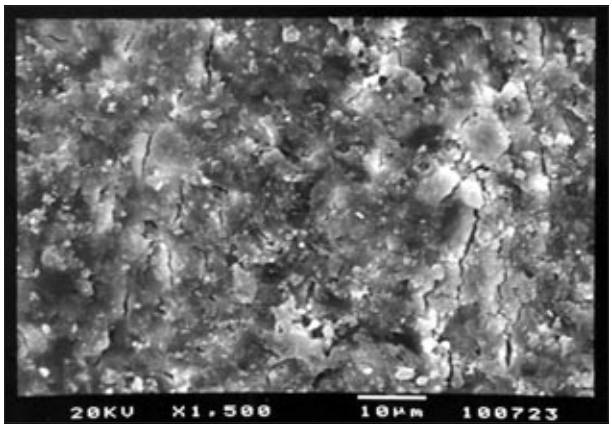


Figure 2 - Control group – the root surface is covered by the smear layer.

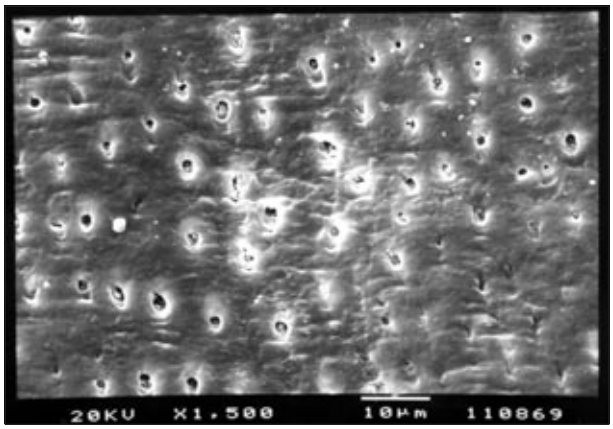
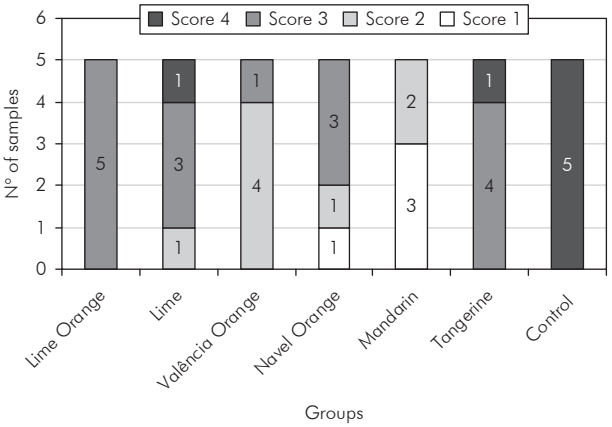


Figure 3 - Photomicrograph of sample treated by friction of valência orange.



Graph 2 - Frequency distribution of scores for the samples of each group that received topical application + friction.

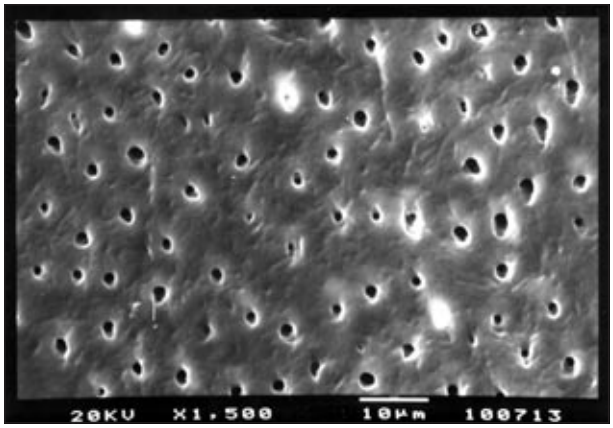
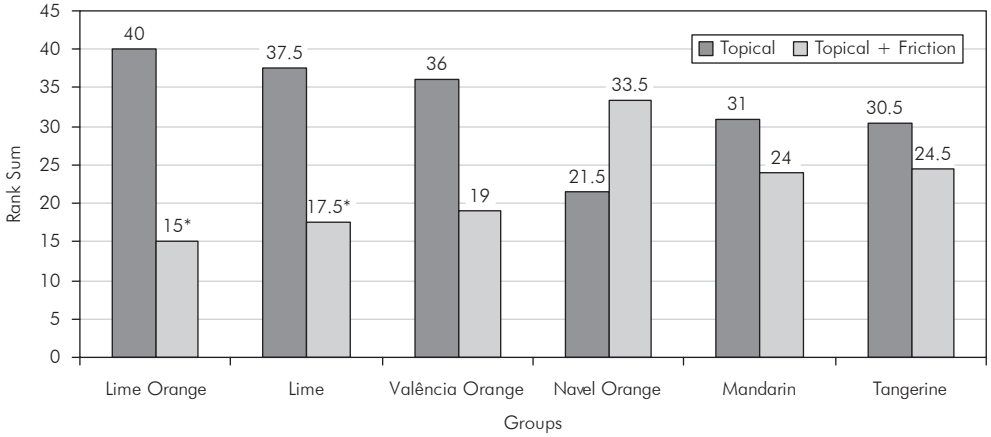


Figure 4 - Photomicrograph of sample treated by friction of mandarin.



Graph 3 - Average rank of natural orange juices. *A comparison between the application methods using the Mann-Whitney test showed significant differences only for the lime orange and lime groups.

comparisons showed that valência orange (Figure 3) and mandarin (Figure 4) were significantly different from the control ($p < 0.05$) (Figure 2).

On the other hand, a comparison between the application methods using the Mann-Whitney test showed significant differences only for the lime or-

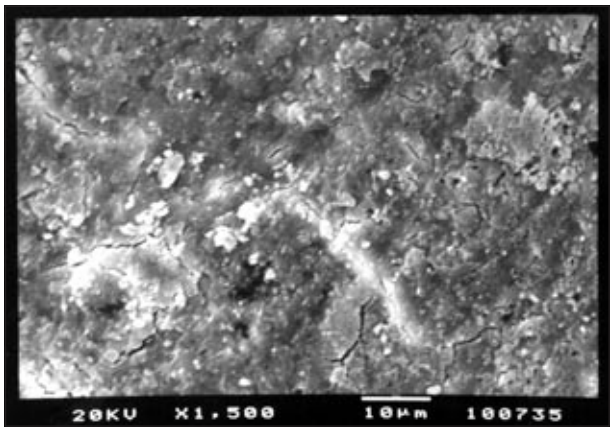


Figure 5 - Lime orange (topical application) – the smear layer is present on the dentin surface and the dentinal tubules are obliterated (Score 4).

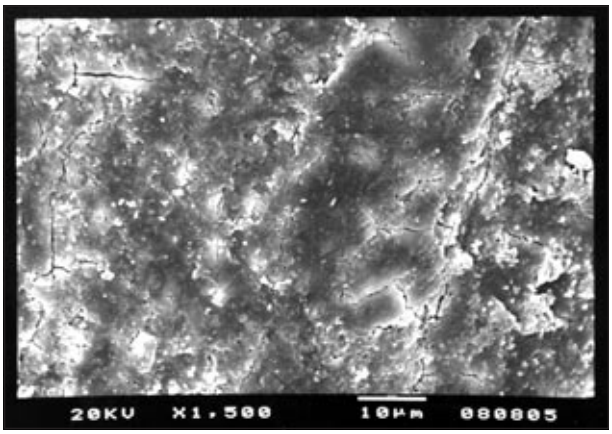


Figure 7 - Lime (topical application) – the smear layer is present on the dentin surface and the dentinal tubules are obliterated (Score 4).

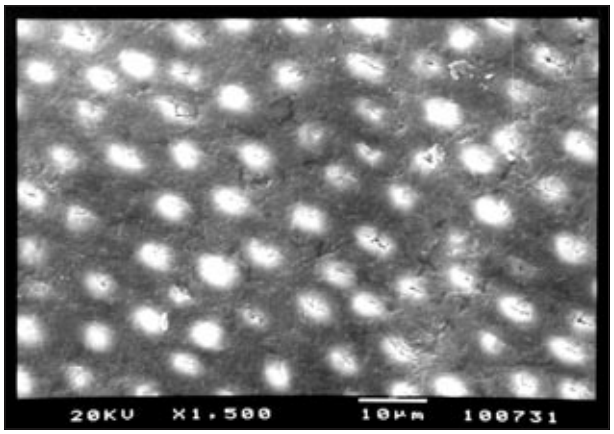


Figure 6 - Lime orange (friction) - indication of dentinal tubules opening (Score 3).

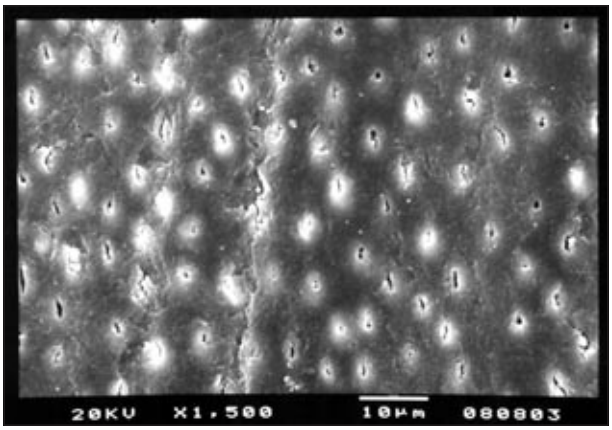


Figure 8 - Lime (friction) - indication of dentinal tubules opening (Score 3).

ange and lime groups (Graph 3). In these groups, the topical + friction application was associated with a lower average rank, which indicates more effectiveness in smear layer removal (Figures 5, 6, 7, 8).

Discussion

Evidence indicates that teeth exhibiting DH have dentinal tubules open at the dentin surface.[4,13] Consequently, the identification of factors which render dentin exposed and tubules open is important for both the prevention and management of this condition. Some factors have been associated with the occurrence of DH, but chemical (erosion) and mechanical (abrasion and attrition) processes are considered to be the most important.

According to Pashley[14] (1992), teeth are not usually hypersensitive immediately after root scaling because the dentinal tubules remain occluded by the smear layer created by instrumentation. However, over time, toothbrushing and acid substances could promote loss of the smear layer, which allows fluid movement in the dentinal tubules in response to stimuli. In this study, the smear layer was created by hand instrumentation with curettes (Figure 2), for subsequent removal by the orange juices.

The erosive effect of dietary acids on tooth tissue can be influenced by many factors including titratable acidity, type of acid, concentration, chelation potential, exposure time, and presence of sugar.[15,16] Moreover, it is well established that the pH is an

Table 1 - Acidity of orange juices immediately after preparation of juice but before application to dentin samples.

Substance	pH
Lime orange	5.27
Lime	5.26
Valência orange	3.35
Navel orange	3.07
Mandarin	2.92
Tangerine	3.17
Control (Distilled water)	5.90

important factor that affects dentinal tubules opening.[16,17] Substances with lower pH can remove the smear layer and open dentinal tubules.[3,6-9] So, it is important to check the substances pH to provide diet recommendations for patients who have DH or dentin exposure. Furthermore, the critical pH at which enamel dissolves is about 5.0 to 5.5, but most fruit juices have a pH below that point.[10] The pH of the natural orange juices tested varied from 2.92 to 5.27 (Table 1).

Vanuspong *et al.*[18] (2002) investigated erosion of dentin by citric acid at different pH values and exposure times, and evaluated whether surface softening or demineralization of dentin occurred, and if so, whether the zone can be remineralized by artificial saliva. Their results showed that erosion of dentin is dependent on time and pH. Some surface softening of dentin occurred during the erosion process and, unlike enamel, dentin showed little propensity to remineralize under ideal conditions *in vitro*.

Previous studies demonstrated that orange juice had an erosive capacity, thus resulting in smear layer removal and dentinal tubules opening.[3,6,9,19] Regarding the importance of dietary counseling to patients and the observed increase in the production and consumption of fruit juices over recent decades, some types of natural orange juices were tested to establish which one has the least effect on dentin surface. Two methods of application were used in the study: Topical application, to simulate the contact between exposed cervical dentin and acidic drinks; and active application, to check if toothbrushing may enhance the erosive effect of dietary acids.[7,12]

In this study, the different types of orange juices tested showed different effects on smear layer removal (Graphs 1, 2). Lime orange (pH 5.27) and lime (pH 5.26) had the highest average rank, similar to that of the control group. These groups were classified predominantly as score 4 (topical) and 3 (topical + friction). Tangerine (pH 3.17) also had a high average rank and it was classified predominantly as score 4 (topical) and 3 (topical + friction). Valência orange (pH 3.35), navel orange (pH 3.07) and mandarin (pH 2.92) had the lowest average rank, which indicates more effectiveness in smear layer removal. However, statistical analysis demonstrated that only navel orange was significantly different from the control group after topical application. On the other hand, after topical + friction, valência orange and mandarin were significantly different from the control.

According to Prati *et al.*[9] (2003), toothbrushing immediately after the exposure of dentin to acidic drinks reduced dentin permeability, creating a new fine and thin smear layer. The authors observed that many tubules were completely opened and free from the smear layer and smear plugs when dentin samples were treated with orange juice. However, after brushing procedures, a small amount of debris was observed on the dentin surface mixed with the smear layer particles.

McAndrew, Kourkouta[20] (1995) investigated the effect of toothbrushing on smear layer formation and evaluated the influence on the patency of dentinal tubules of toothbrushing preceded and/or followed by orange juice application. The results showed that toothbrushing alone was the most effective in occluding the tubules, followed by toothbrushing subsequent to dietary acid application, and then by toothbrushing prior to acid application. Based on these results, in cases of DH toothbrushing should not precede or follow dietary acid application, but be separated from mealtimes. The Canadian Advisory Board on Dentin Hypersensitivity[21] recommended that patients at risk of erosion or abrasion should be advised to brush their teeth before meals.

The Mann-Whitney test demonstrated a significant difference between the application methods

only for lime orange and lime (Graph 3). These groups showed the highest values of pH (Table 1) and resulted in complete obliteration of dentinal tubules without smear layer removal after topical application (Graph 1). However, the toothbrushing in the active application removed the smear layer partially and resulted predominantly in score 3 (Graph 2). Although statistical difference has not been found in the other groups, there was a reduction of the average rank after active application, except for navel orange, which indicates more effectiveness in smear layer removal (Graph 3). These data suggest that toothbrushing tends to increase smear layer removal. Previous studies observed that toothbrushing without dentifrice in the presence of acids accelerated the process of erosion and tubule opening.[6,8]

DH is a common problem found in adult populations and a large number of different treatments have been employed in the management of this condition, but so far no single universally accepted treatment is available. However, it is conceivable that prevention and treatment of DH depend on the control of the patient's dietary habits and toothbrushing behavior.[6,10,11,21]

Some caution must be exercised in extrapolating the findings of this *in vitro* study to the clinical condition, but the consumption of navel orange, valência orange and mandarin many times a day might be associated with occurrence of DH. Thus, it is reasonable to recommend to patients with exposed dentin that, rather than avoiding orange juice consumption, they could replace these types of orange juices with highest erosive capacity by others that have less or no effect on the root surface such as lime orange and lime.

Conclusion

According to the methodology proposed and based on the results of this study, the following conclusions may be drawn:

1. Navel orange, valência orange and mandarin are more effective in terms of smear layer removal and dentinal tubules exposure in comparison with lime orange and lime.
2. Toothbrushing without dentifrice tends to improve smear layer removal after orange juice application, except for navel orange.

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