Evaluation of a new hardness tester (Cariotester): Comparison with transverse microradiography for assessing the inhibitory effect of fluoride application on bovine root dentin demineralization

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The objective of this study was to investigate the correlation between CT depth, indentation depth determined by a new hardness tester (Cariotester), and the transverse microradiography (TMR) parameters, i.e., lesion depth and mineral loss. For that purpose, this study evaluated the feasibility of using Cariotester as a root caries diagnostic system and capability of Cariotester to detect effect of fluoride application on inhibiting dentin demineralization. Fluorides were applied to bovine root dentin specimens, which were subsequently demineralized for 1–21 days and then CT depth and TMR parameters were assessed. There were significant correlations between CT depth and TMR parameters in fluoride and non-fluoride groups. There were significant differences between fluoride and non-fluoride groups for CT depth and TMR parameters respectively. Current results suggested that Cariotester may be capable of providing an objective evaluation of root caries progression and the fluoride effect on inhibiting dentin demineralization.

Keywords: Cariotester, Dentin demineralization, Root caries, Fluoride, TMR

INTRODUCTION

Increasingly aging populations have become a global issue in recent years. A United Nations report has predicted that by 2050, the proportion of the world’s total population aged 60 or over will rise to 21.2%1. This increase in the number of elderly people poses numerous health problems that must be overcome by clinical institutions. One of the problems in dentistry is dealing with root caries. Preventing and arresting root caries is one of the essential issues to maintain the quality of life of elderly people.

Advances in dental care, increased numbers of dentists and dental hygienists, the addition of fluoride to toothpaste, and better awareness among elderly people of the importance of preserving their own teeth have all helped to increase the number of teeth in elderly people who still have their own teeth over the past few decades2,3. The prevalence of root caries, however, is rising along with the age of elderly people4,5. These facts mean that root caries will inevitably be encountered more frequently in clinical practice in future.

Tooth root minerals are more vulnerable to demineralization than enamel6. When root caries extends below the gingival margin, moisture control of the prepared cavity is difficult and as a result, the success rate of restoration diminishes7. This means that once root caries with a cavitation has formed, it is often impossible to treat it with conventional restorative treatment. Efforts toward prevention should therefore be the foremost priority.

Fluoride application is an established method of preventing root caries. However, there is as yet no generally established diagnostic method for root caries with objective quantification.

Existing methods of diagnosing root caries that have been considered include visual inspection and clinical probing8, quantitative light-fluorescence (QLF™, Inspektor Research systems, Amsterdam, The Netherlands)9, laser fluorescence (DIAGNOdent™, KaVo, Biberach, Germany)10, and measurement of electrical impedance using an electric caries meter (ECM III, Lode Diagnostics BV, Groningen, The Netherlands)10. A number of problems remain, however, with all these methods. Although visual inspection and clinical probing are the most commonly used techniques, inter-examiner diagnostic consistency is only moderate9, and it has problems with objectivity. The QLF system detects porphyrin-derived fluorescence to evaluate the degree of dentin demineralization11. It is excellent for objective detection of root caries itself9, but still has problems with the quantitative determination of its stage of development, and further studies are required. The DIAGNOdent is said to be capable of distinguishing between active and inactive root caries according to the measured values. However, the correlation between DIAGNOdent values and the progression of root caries is insufficiently strong10. ECM provides good inter-examiner reproducibility, but similar to the DIAGNOdent, the correlation between ECM values and root caries progression is not strong enough10.

In 2009, Shimizu et al.12 developed the Cariotester (SUK-971, SaneiME, Yokohama, Japan) as a hardness tester for the tooth structure, and it was used in studies
by Utaka et al., and Iwami et al. The Cariotester system is a small, portable device that uses a simple measurement principle and is easy to operate. The hardness is calculated from the depth to which the indenter presses into the tooth surface. It also has the other advantages that one measurement only takes around 10 s to perform, it can be used to measure hardness on non-flat surfaces, and can be used intra-orally. The Cariotester is the world’s first hardness tester that can be used at the chairside. Featherstone et al. reported that there is a linear relationship between the square root of the Knoop hardness of demineralized dentin and mineral density measured by transverse microradiography (TMR), which is regarded as the gold standard. This suggests that it may be possible to use Cariotester hardness measurements to evaluate mineral density in carious dentin. From a similar perspective, Utaka et al. compared the TMR parameters of lesion depth (LD) and mineral loss (AZ) with the indentation depths (CT depth) obtained when using the Cariotester in root caries to investigate the remineralizing effect of fluoride in vitro. They found that there were significant correlations between TMR parameters and CT depth. Caries develops with cyclic processes of de- and remineralization. Thus a new diagnostic system must be proved to have reliability for mineral assessment occurring de- and remineralization.

The objectives of this study were (1) to investigate the correlation between CT depth and TMR parameters, in order to investigate the feasibility of using the Cariotester as a root carries diagnostic system, i.e. whether it fulfills criterion-related validity; (2) to determine whether or not the Cariotester is capable of detecting the effect of fluoride application on inhibiting dentin demineralization; and (3) to evaluate the intraclass correlation (repeatability of measurements) of CT depth. In this study, we used sodium fluoride (NaF) and acidulated phosphate fluoride (APF), which are the fluorides most frequently used in daily practice.

MATERIALS AND METHODS
Preparation of root dentin specimens
Bovine teeth that had been frozen after extraction were thawed, and one quarter from the apical side of root and coronal part of the bovine teeth were removed. A total of 150 blocks measuring approximately 2.0×3.0×2.0 mm were prepared from the root dentin, and embedded in resin (GC Ostron RII, GC, Tokyo, Japan). The window sides of the embedded blocks were ground flat using SiC waterproof abrasive paper of 800–1,000 grid. An SS-OCT system (Santec OCT-2000®, Santec Co., Komaki, Japan) was then used to confirm removal of the cementum and exposure of the dentin surface. All of the dentin surface outside a 1.5×2.5 mm area was covered with nail polish to create the treatment window.

Fluoride application and demineralization
Figure 1 shows a flow chart of the experimental procedure. Of the 150 specimens, 50 were treated with a neutral pH solution of sodium fluoride (NaF) (Neo Sodium Fluoride Solution, Neo Dental Chemical Products, Tokyo, Japan; 2% NaF), and 50 with acidulated phosphate fluoride (APF) (Fluor Solution Dental 2%, Oriental Pharmaceutical and Synthetic Chemical, Osaka, Japan; 2% NaF). The remaining 50 specimens that were not treated with fluoride served as controls (no fluoride treatment). Five μL of the fluoride solution concerned was dropped onto the specimen surfaces in the NaF and APF groups and left for 5 min, after which the specimens were rinsed with distilled water and the remaining water was wiped off. The specimens were then immersed in demineralizing solution (2.2 mM CaCl₂, 2.2 mM KH₂PO₄, 50 mM CH₃COOH, 3.1 mM NaN₃, pH 5.0) and 10 specimens in each group were left in the demineralization solution at a constant temperature of 37°C for 1, 3, 7, 14, and 21 days each. The demineralization solution was replaced with fresh solution at 7-day intervals.

Measurement of indentation depth using the Cariotester system
The Cariotester consists of 3 parts: a handpiece with an arm with an indenter attached, a digital microscope, and a laptop computer (Fig. 2). The handpiece is fitted with an indenter for measuring indentation hardness at the tip. The tip of the indenter is a cone with a conical angle of 50°, and the tip of the cone is spherical with a diameter of 18 μm. Before measurements were made, the tip of the indenter was coated with white poster paint. The indenter was then fixed to the arm, and pressed into the specimen surfaces. In the process, it was confirmed that the angle between a line perpendicular to the axis of the indenter and the disappearance line of the paint was below 15°. A buzzer was set to ring when the load reached 150 gf 1.0 s after indentation, and the indentation was halted at the same time.
After measurement, the arm fitted with the indenter was detached from the handpiece and fixed in a V-shaped groove in the stand supplied with the microscope. The tip of the indenter was magnified by the microscope and displayed on the screen of the laptop computer. The indentation depth was defined as the distance between the tip of the indenter and the disappearance line. The depth was measured at three well-separated and randomly selected sites on the window surface, and their average measurement was taken as the indentation depth of the specimen (CT depth: μm). The CT depth of all 150 specimens was measured before demineralization, and the specimens were allocated to 15 groups (3 treatment conditions×5 durations of immersion in demineralization solution) in such a way that the mean baseline CT depth for each group was almost the same at 56±9 μm. The CT depths of all the specimens were measured again after 1, 3, 7, 14 and 21 days of immersion in demineralization solution. During these measurements, after the specimens were removed from the demineralization solution they were lightly sprayed with air by using a dental air blower and measurements were made within 3 min to prevent the window surface from drying out.

**TMR analysis**

After CT depth had been measured, the specimens were sliced with a low-speed diamond saw (Isomet 5000; Buehler, Lake Bluff, Illinois, USA) into sections of thickness 220 μm. After cutting, the sections were immediately immersed in 70% aqueous glycerin solution to prevent them from drying out. Immediately before TMR imaging, the sections were taken out of the glycerin solution and the excess solution was removed. These sections and a 15-step aluminum step wedge were then placed on a x-ray glass plate (High Precision Photo Plate; Konica Minolta Photo, Tokyo, Japan), and a soft x-ray generator (SOFTEX CMR-2; Softex, Kanagawa, Japan) was used for TMR imaging under the conditions of tube voltage 20 kV, tube current 2.50 mA, and exposure time 10 min. After imaging, the glass plate was developed in 5 min at 20°C, fixed in 3 min at 20°C, washed, and dried according to the standard procedure. After drying, it was digitally photographed using a microscope (SMZ1000, Nikon Corp., Japan) and CCD camera (DS-Fi1, Nikon Corp., Tokyo, Japan) and the images were analyzed by using image analysis software (Image J; version 1.42q, Wayne Rasband, NIH, USA) and customized image processing software to calculate LD (μm) and ΔZ (vol.%·μm).

**Statistical analysis**

As interactions between CT depth and TMR parameters (LD and ΔZ) were evident in the data for each group in the control, NaF, and APF groups, we carried out statistical analysis by using one-way analysis of variance and Dunnett’s T3 multiple comparison test ($\alpha=0.05$) with the three groups and duration of demineralization as independent variables and CT depth as a dependent variable. We analyzed CT depth and TMR parameters by comparing them between the various durations of demineralization in the three groups, and between the three groups for the same duration of demineralization. The correlation between CT depth and TMR parameters was investigated in terms of Spearman’s rank-order correlation coefficient ($\rho$) with 95% confidence intervals both separately for each group and with the pooled data from all three groups. We also analyzed the repeatability of measurements within groups in terms of the intraclass correlation coefficient by using the 10 specimens in each of the 3 groups that were demineralized for the same amount of time. SPSS version 19.0 Chicago, Illinois, USA was used for statistical analysis.

**RESULTS**

All the test and control groups showed an equivalent initial (pre-demineralization) CT depth, indicating the specimen allocation to the individual group was well organized. Figure 3 shows representative TMR images in the control, NaF and APF groups. The lesions in the NaF and APF groups were subsurface lesions, with a mineralized layer showing relatively higher mineral density on the lesion surface, but not those in the...
control group.

Figure 4-a shows the changes over time in CT depth in each group. Time is shown as square root (√t) of the duration (day) of demineralization (all changes over time in this study are expressed as √t). Approximation formulae relating CT depth and √t were derived, and their correlation coefficients (r) were calculated. The results were highly linear in all the groups at 0.875–0.964. Table 1 shows the mean and SD values of CT depth and TMR parameters in the experimental groups for different demineralization period and statistical significances among the groups. In the control and NaF groups, there was no significant difference between the specimens at 14 and 21 days, but significant differences were apparent between all the other durations of demineralization. In the APF group, there were no significant differences between the specimens at 3 and 7 days or between those at 7 and 14 days, but significant differences were apparent between all the other durations of demineralization. In the intergroup comparison, there were significant differences between the control group and the fluoride groups in all durations of demineralization. There were also significant differences between the NaF and APF groups in all durations of demineralization, with CT depths being smaller in the APF group than in the NaF group.

Figure 4-b shows the changes in LD over time. The correlation coefficients (r) of the approximation formulae for the 3 groups were in the range 0.593–0.912. The values of r were lower for the NaF and APF groups (0.726 and 0.593, respectively) than the control group (0.912). Table 1 shows significant differences within the groups. In the control group, significant differences were apparent between all the durations of demineralization other than between 3 and 7 days and between 14 and 21 days. In the NaF group, although the difference between 21 and 14 days was not significant, there were significant differences between 21 and the other durations of demineralization. In the APF group, although the difference between 1 and 3 days was not significant, there were significant differences between 1 day and the other durations of demineralization. In the intergroup comparison, there were significant differences between the control group and the fluoride groups for any duration of demineralization longer than 7 days. There were no significant differences in the control group.
Table 1  Statistical analysis of CT depth and TMR parameters (LD, ∆Z)

a: Means and standard deviation in CT depth (μm)

<table>
<thead>
<tr>
<th>Demineralization time</th>
<th>Control</th>
<th>NaF</th>
<th>APF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-day</td>
<td>97 (9)</td>
<td>76 (8)</td>
<td>65 (9)</td>
</tr>
<tr>
<td>3-day</td>
<td>161 (9)</td>
<td>104 (10)</td>
<td>86 (8)</td>
</tr>
<tr>
<td>7-day</td>
<td>218 (35)</td>
<td>119 (10)</td>
<td>92 (7)c,d</td>
</tr>
<tr>
<td>14-day</td>
<td>323 (25)a</td>
<td>137 (9)b</td>
<td>100 (9)d</td>
</tr>
<tr>
<td>21-day</td>
<td>352 (34)a</td>
<td>162 (24)b</td>
<td>126 (13)</td>
</tr>
</tbody>
</table>

b: Means and standard deviation in LD (μm)

<table>
<thead>
<tr>
<th>Demineralization time</th>
<th>Control</th>
<th>NaF</th>
<th>APF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-day</td>
<td>94 (42)a</td>
<td>136 (46)A,c</td>
<td>108 (60)A,e</td>
</tr>
<tr>
<td>3-day</td>
<td>190 (63)Ra</td>
<td>141 (36)Rc</td>
<td>173 (67)Ra,if</td>
</tr>
<tr>
<td>7-day</td>
<td>237 (32)c</td>
<td>161 (32)C,c,d</td>
<td>191 (34)c,Fi</td>
</tr>
<tr>
<td>14-day</td>
<td>333 (48)c</td>
<td>208 (59)D,c,d,</td>
<td>205 (40)c,Fi</td>
</tr>
<tr>
<td>21-day</td>
<td>374 (27)c</td>
<td>279 (48)E,d</td>
<td>227 (48)c,Fi</td>
</tr>
</tbody>
</table>

c: Means and standard deviation in ∆Z (vol%•μm)

<table>
<thead>
<tr>
<th>Demineralization time</th>
<th>Control</th>
<th>NaF</th>
<th>APF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-day</td>
<td>1,536 (489)</td>
<td>447 (217)A</td>
<td>452 (329)A,e</td>
</tr>
<tr>
<td>3-day</td>
<td>3,386 (1117)</td>
<td>1,102 (398)B,c</td>
<td>808 (439)B,e,f</td>
</tr>
<tr>
<td>7-day</td>
<td>5,573 (1046)Ra</td>
<td>1,339 (807)C,r,d</td>
<td>771 (368)c,F</td>
</tr>
<tr>
<td>14-day</td>
<td>8,076 (2290)c,b</td>
<td>1,604 (988)c,d</td>
<td>1,337 (680)c,F</td>
</tr>
<tr>
<td>21-day</td>
<td>8,544 (1597)c</td>
<td>2,613 (989)c,d</td>
<td>1,573 (861)c,F</td>
</tr>
</tbody>
</table>

In each row, differences between values marked with the same superscript uppercase letter are not significant (p>0.05).
In each column, differences between values marked with the same superscript lowercase letter are not significant (p>0.05).

between the NaF and APF groups.

Figure 4-c shows the changes in ∆Z over time. The values of r for the approximation formulae for the control, NaF, and APF groups were in the range 0.581–0.879. The values of r were lower for the NaF and APF groups (0.694 and 0.581, respectively) than the control group (0.879). Table 1 shows the significant differences within the groups. In the control group, there were no significant differences between the specimens at 7 and 14 days or between those at 14 and 21 days, but there were significant differences between all the other durations of demineralization. In the NaF group, there were significant differences between the specimens at 1 day and all other durations of demineralization, as well as between 3 and 21 days. In the APF group, the only significant differences were between 1 and 14 days and between 1 and 21 days. In the intergroup comparison, there were significant differences between the control and fluoride groups in all durations of demineralization. There were no significant differences between the NaF and APF groups.

The mean rates of inhibition of demineralization throughout all the demineralization periods were calculated from the slopes of the approximation formulae in Fig. 4 by using the expression below.

Mean rate of inhibition of demineralization (%)=\left[1−\text{(slope of approximation formula for fluoride group)/slope of approximation formula for the control group)}\right]×100

This showed that in terms of CT depth, demineralization was inhibited by 70% in the NaF group and by 80% in the APF group. In terms of LD, it was inhibited by 49% in the NaF group and by 62% in the APF group, while in terms of ∆Z it was inhibited by 74% in the NaF group and by 85% in the APF group. In all cases, fluoride was shown to be effective in inhibiting demineralization compared with the control group.

Figures. 5-a and -b shows correlation diagrams between CT depth and LD or ∆Z. Table 2 shows the correlation coefficients (ρ), confidential intervals (CI),
Fig. 5 Relationship between CT depth and TMR parameters (LD and ∆Z).
□: Control group, ●: NaF group, ○: APF group. a: LD □: y=1.0x+22.6 (r=0.881), ●: y=1.4x+11.3 (r=0.668), ○: y=1.5x+37.3 (r=0.525). b: ∆Z □: y=26.2x−614.1 (r=0.861), ●: y=22.3x−1219.1 (r=0.708), ○: y=16.7x−576.7 (r=0.531).

Table 2 Spearman’s rank-order correlations coefficient (ρ) between CT depth and TMR parameters (LD, ∆Z) with 95% confidence intervals.

<table>
<thead>
<tr>
<th>Correlations between CT depth and TMR parameters</th>
<th>ρ</th>
<th>p-value</th>
<th>95% CI for ρ</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD 3 groups combined</td>
<td>0.681</td>
<td>&lt;0.01</td>
<td>0.585–0.759</td>
</tr>
<tr>
<td>∆Z 3 groups combined</td>
<td>0.843</td>
<td>&lt;0.01</td>
<td>0.789–0.884</td>
</tr>
<tr>
<td>LD Control group</td>
<td>0.860</td>
<td>&lt;0.01</td>
<td>0.765–0.919</td>
</tr>
<tr>
<td>LD NaF group</td>
<td>0.706</td>
<td>&lt;0.01</td>
<td>0.532–0.823</td>
</tr>
<tr>
<td>LD APF group</td>
<td>0.519</td>
<td>&lt;0.01</td>
<td>0.281–0.697</td>
</tr>
<tr>
<td>∆Z Control group</td>
<td>0.846</td>
<td>&lt;0.01</td>
<td>0.743–0.910</td>
</tr>
<tr>
<td>∆Z NaF group</td>
<td>0.756</td>
<td>&lt;0.01</td>
<td>0.605–0.855</td>
</tr>
<tr>
<td>∆Z APF group</td>
<td>0.527</td>
<td>&lt;0.01</td>
<td>0.291–0.702</td>
</tr>
</tbody>
</table>

and p-values separately for the three groups and for the three groups pooled together. The correlation coefficients between them were all comparatively high (0.519–0.860).

When the repeatability of measurements within groups was analyzed in terms of the intraclass correlation coefficient by using 10 specimens in each of the three groups that were demineralized for the same amount of time, the Cronbach’s α value was 0.99, 0.98 and 0.98 for the control, NaF and APF group, respectively, and was even higher when the three groups were pooled, at 1.0.

**DISCUSSION**

Many previous studies have used TMR as the gold standard for evaluating the progress of demineralization and remineralization. We therefore analyzed the association between CT depth and the TMR parameters of LD and ∆Z (Table 2).

For LD, the correlation between CT depth and LD was greatest (ρ=0.860) in the control group when the 3 groups were treated separately, and the slope of the correlation formula was 1.0 (Fig. 5). This means that the measured values for CT depth and LD were almost consistent. Similarly for the fluoride groups, the correlation was high (r=0.706 for NaF and r=0.519 for APF). While the slopes of the correlation formulae in the fluoride groups were 1.4 for the NaF group and 1.5 for the APF group, meaning that the CT depth was approximately 71% of LD for the NaF group (1.0/1.4) and approximately 67% (1.0/1.5) for the APF group. This difference may have been because in the fluoride groups the lesion surface was covered with a relatively higher mineralized layer, which inhibited indentation by the indenter. When the specimens in all three groups were pooled, the correlation coefficient between CT depth and LD was comparatively good (r=0.681).

For ∆Z, the correlation coefficient r was 0.843 when specimens from all three groups were pooled, higher than for LD. The reason may have been that as described above, CT depth directly reflects the hardness
of the dentin, and hardness itself is correlated with mineral density\cite{15-17}. When the three groups were treated separately, similarly to LD, the $\rho$ value for the control group was 0.846, higher than the values of 0.756 for the NaF group and 0.527 for APF group.

CT depth was validated by TMR as shown in the discussion above, and CT depth exhibited significant differences at all durations of demineralization (except 14- and 21-day) in the control group (Table 1), those sufficiently fulfill the criterion-related validity. This suggests that the Cariotester may be capable of appropriately monitoring the progression of dentin demineralization.

Utaka et al. evaluated the effect of fluoride in dentin lesion remineralization by using the Cariotester and TMR and investigated the correlation between CT depth and LD or $\Delta Z$, finding that there was a significant correlation between them\cite{21}. Their results were consistent with our findings in this study that the Cariotester can be used to evaluate mineral density in root dentin.

In this study, we analyzed the data by plotting the degree of demineralization (CT depth, LD, $\Delta Z$) against the square root of the duration of demineralization. We found linearity with relatively high low coefficient of determination ($r=0.581$–0.964) in all cases. This was consistent with previous studies showing the existence of linear relationships between the square root of the duration of demineralization and various parameters for quantifying the degree of demineralization (elution of calcium ions or phosphate ions) when specimens are demineralized under consistent demineralization conditions\cite{21}.

It is widely known that fluoride inhibits dentin demineralization and arrests caries progression\cite{18,22,23,29}. In the present investigation, significant differences in CT depth between both fluoride and control groups were evident after the first day of demineralization (Table 1). The fact that the Cariotester was able to detect the effect of fluoride in inhibiting demineralization compared with the control group, i.e., demonstration of discriminant validity suggests that it can be used in clinical trials to assess the fluoride efficacy.

In root caries, collagen is exposed and enzymatically degraded after demineralization, and this is a major difference from enamel caries\cite{24}. A number of studies have addressed the effects of collagen degradation on lesion progression and remineralization, and animal experiments have shown that suppressing the enzymatic degradation of collagen can inhibit root caries progression\cite{25,26}, but the role of collagen degradation in dentin caries progression and remineralization is still not fully explained. In this context, it is highly plausible that the degree of collagen degradation may affect CT depth, and this point requires future investigation.

It has recently been reported that silver diamine fluoride (SDF) is more effective in preventing root caries than are NaF varnish and chlorhexidine\cite{27}. Sugawara et al.\cite{29} carried out an in vitro study using the Cariotester to evaluate the effect of SDF in inhibiting dentin demineralization. They found significant correlation between CT depth and LD, but reported that they were unable to discuss any correlation with $\Delta Z$. This was because the silver ions in SDF have higher radio-opacity compared with that of Ca in dentin, and their deposition on the tooth surface creates the appearance of pseudo-mineralization on TMR images. This phenomenon hinders the evaluation of mineral density on TMR images. When evaluating the effect of SDF in vitro, the Cariotester may serve as a useful substitute for TMR.

**CONCLUSION**

This in vitro study revealed a high correlation between CT depth and TMR parameters (LD and $\Delta Z$) in dentin lesion, indicating that CT depth was suitable for use in monitoring root dentin demineralization over time, and was also capable of detecting the effect of fluoride agents (NaF and APF) in inhibiting demineralization. There is intraclass correlation (repeatability of measurements) of CT depth. These results were achieved thanks to the extremely high repeatability of the CT measurement, suggesting that the Cariotester may be capable of providing an objective evaluation of root caries progression and the effect of fluoride on inhibiting demineralization.

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