Assessment of root caries under wet and dry conditions using swept-source optical coherence tomography (SS-OCT)

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The purpose of this study was to compare optical properties of root caries under two observing conditions using swept-source optical coherence tomography (SS-OCT). In vitro natural and root caries were observed by SS-OCT under wet and dry conditions, followed by confocal laser scanning microscope (CLSM) and transverse microradiography (TMR). Signal intensity (SI), distance between SI peaks (SI-distance) and optical lesion depth were obtained from OCT. Lesion depth was measured from CLSM; lesion depth (LDTMR) and mineral loss (ML) were obtained from TMR. In vitro root caries under wet and dry conditions showed different OCT images and SI patterns. Under dry conditions, half natural root caries showed similar OCT images and SI patterns as in vitro root caries. The base of demineralized dentin could be detected more clearly under dry conditions than under wet conditions.

Keywords: Root caries, Optical coherence tomography, Biofilms, Streptococcus mutans

INTRODUCTION

Root caries usually occurs where root dentin is exposed to oral environment as a result of gingival recession, which may be caused by mechanical toothbrush trauma or chronic periodontitis11. Many host factors affect root caries initiation and progression. Those factors include biofilm and saliva characteristics, immune system response, access to fluoride and diet22. Bacterial acidification not only induces demineralization and exposure of the organic matrix in dentin and root surfaces but also activation of dentin-embedded and salivary matrix metalloproteinases (MMPs) and cathepsins, which can initiate a degradation of exposed demineralized organic materials9. Cementum and dentin surfaces are more susceptible to carious attack than enamel surfaces due to their lower degree of mineralization9. Root caries increases in prevalence with age and will participate the breakdown of remaining natural and restored teeth5. They may also compromise the long-term success and survival of periodontally treated teeth6.

Early diagnosis of root caries and dentin lesions may allow the dentists to implement non-invasive strategies to reverse the lesion and to avoid surgical interventions. Current methods for lesion assessment are composed of visual and tactile exams, which are prone to subjective bias and interference from staining7. Based on the changes in optical properties between healthy and caries teeth, optical coherence tomography (OCT) has become one of the caries detection tools8. OCT is a noninvasive diagnostic method for obtaining cross-sectional images of internal biological structures9. It has been widely used in assessment of demineralization based on two main principles: increased light scattering in the porous demineralized tissue and depolarization of the incident light by the demineralized tissue. The latter necessitates a polarization-sensitive OCT (PS-OCT) or cross-polarization OCT (CP-OCT)10-12, but the former phenomenon can be observed as increased signal intensity by both conventional and polarized-sensitive OCT systems. Swept-source OCT (SS-OCT) system has been developed as an implementation of spectral discrimination, in which the laser light source swept the near-infrared wavelength at a high rate13. Because SS-OCT has higher sensitivity and specificity than radiographic methods, it has been used to diagnose non-carious cervical lesions in vivo14 and to assess cervical dentin demineralization in vitro15.

Recent studies, using conventional OCT without polarization sensitivity, have pointed out the effect of hydration on OCT images8,16. As water within the porosities of the demineralized dental tissues reduced

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the diffused scattering within the lesion which was caused at micro-interfaces between crystals and air, a visual lesion boundary was clearer under hydrated conditions than that under dry conditions in OCT studies on assessment of early lesions\(^{13,16,17}\). To our knowledge, there are few studies demonstrating OCT without polarization sensitivity can be used to compare the optical properties of root caries under wet and dry conditions.

Image analysis techniques in correlative OCT studies have been mainly based on the increased signal intensity values to quantify parameters such as depth and mineral loss in demineralized lesions\(^{13}\). The aim of this study was to observe and compare optical properties of both \textit{in vitro} and natural root caries under controlled wet and dry conditions using SS-OCT. Also the correlation was examined between OCT data and lesion parameters, including lesion depth and mineral loss, determined by common techniques such as confocal laser scanning microscope (CLSM) and transverse microradiography (TMR). The null hypotheses were: (1) root caries under wet and dry conditions showed the same OCT images and optical properties; (2) \textit{in vitro} and natural root caries showed the same OCT images and optical properties.

**MATERIALS AND METHODS**

**Specimen preparation**

After obtaining the informed consent according to a protocol approved by the Ethical Review Board for the Usage of Human Teeth, Human Research Ethics Committee, Tokyo Medical and Dental University (approval No. 725), 4 intact human premolars and 6 human premolar teeth with root caries were selected (Fig. 1, Table 1). The teeth were stored in 4°C in water containing 0.1wt% of thymol.

The schematic diagram of specimen preparation of

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**Table 1** Criteria of natural root caries selection in this study

| Criteria                  | Content                                                                 |
|---------------------------|-------------------------------------------------------------------------|
| ICDAS II root caries      | There is a clearly demarcated area on the root surface or at the cemento-enamel junction that is discolorated (light/dark brown, black) and there is cavitation (loss of anatomical contour ≥0.5 mm) present. |
| Activity: not active      | 1. Examination: visual and tactile                                       |
|                           | 2. Characteristics: texture (smooth, rough), appearance (shiny or glossy, matte or non-glossy) and perception on gentle probing (soft, leathery, hard) |
|                           | 3. Activity: active root caries lesions are usually located within 2 mm of the crest of the gingival margin |
the 4 intact premolars was shown in Fig. 1-a. The cervical parts were cut by a low-speed diamond saw (IsoMet 5000, Buehler, Lake Bluff, IL, USA) and vertically cut into half. Eight square-shaped blocks (5×4×2 mm) were made by a diamond bur (FG 102R, Shofu, Kyoto, Japan) attached to an air turbine hand-piece under copious cooling water. Two semi-round notches (depth: 0.5 mm) were marked by a diamond bur (D4010f, GC, Tokyo, Japan) on the top surface. The specimens were observed by SS-OCT (IVS-2000, Santec, Komaki, Japan). Then five cutting surfaces of each specimen were covered with a thin layer of acid-resistant varnish (Shiseido, Tokyo, Japan) and one edge of the root top surface was covered by 1-mm-wide paraffin wax (GC) to prevent demineralization.

Each of the 6 human premolar teeth with natural root caries was marked a 5-mm-long line by pencil on the top central surface (Fig. 1-b). The bottom was fixed by red utility wax (GC) on a glass plate. They were utilized for direct OCT observation across the marked lines according to the experiment procedures as mentioned below.

**In vitro root caries formation**

In vitro root caries on the 8 tooth cervical blocks from the 4 intact human premolars was fabricated using the biofilm-induced demineralization method twice (Fig. 1-a). Each time, in vitro biofilms were formed on the tooth surfaces using a laboratory strain of oral cariogenic bacteria *Streptococcus mutans* (*S. mutans*) MT8148. The biofilms were formed in an oral biofilm reactor (OBR) and then incubated for 3 days\(^{18,19}\). After demineralization, the specimens were observed by SS-OCT. The process was labeled as Dem0 (before demineralization), Dem1 (after the 1st demineralization) and Dem2 (after the 2nd demineralization).

**Swept-source optical coherence tomography (SS-OCT) observation and measurement**

SS-OCT was used to examine in vitro and natural root caries under wet and dry conditions. The wavelength ranged from 1,260 to 1,360 nm (centered at 1,310 nm) at a 20-kHz sweep rate. The axial resolution of the system is 12 µm in air, which corresponds to 8 µm in tissue assuming a refractive index of approximately 1.5. The lateral resolution is 20 µm. A 2,004×1,000-pixel 2D cross-sectional image (5×7.481 mm) can be created by converting the raw data into a grey scale digital image. The SS-OCT probe was set on a holder at a fixed distance from the specimen with the scanning beam oriented perpendicular with respect to the root surfaces. The 8 tooth blocks with in vitro root caries were observed following the scanning procedure: Dem0, Dem1 and Dem2. Then the 6 teeth with natural root caries were observed. First, the specimens were cross-sectionally scanned under wet conditions (observing after gentle blot-drying of the surface, leaving it moist without any visible water-droplets)\(^{17}\). Then, the specimens were scanned under dry conditions (observing after keeping the specimens in air at room temperature for 1 h). In order to be able to scan the same location repeatedly, the scanned areas were conducted across the two marked notches every time.

A custom code in the image analysis software (Image J, version 1.48, National Institutes of Health, Bethesda, MD, USA) was used to read the raw data of SS-OCT. The linear measurements of lesion depths were shown in Figs. 2-a and -a’. As dentinoenamel junction (DEJ) was not clear after demineralization, the lesion depths were measured from the lesion surface rather than DEJ to the lesion boundary. The lesion depths of 20 positions were measured with a width of 2,000 µm and an interval of 100 µm. The average was calculated as the value of lesion depth of one specimen. The lesion depths
Fig. 3 Signal intensity profile and mineral intensity profile of in vitro root caries. (a) From OCT image of Dem2 under dry conditions, an area of interest with an optical depth of 2,000 µm and a width of 1,000 µm was selected. (b) The horizontal distance between two signal intensity peaks (SI-distance) after Dem2 under dry conditions was measured. (c) From TMR image, an area of interest with a depth of 2,000 µm and a width of 1,000 µm was selected. (d) The lesion depth of TMR (LD_{TMR}) and the mineral loss (ML, vol.%) were obtained by software automatically. The red broken line shows a baseline, where the mineral content of dentin is calculated considering the sound area is containing 47 vol.% mineral content.

were labeled as LD_{1OCTwet} (Dem1), LD_{1OCTdry} (Dem1), LD_{2OCTwet} (Dem2) and LD_{2OCTdry} (Dem2). The signal intensity (SI) and the horizontal distance between two SI peaks (SI-distance) of Dem2_{dry} were investigated (Figs. 3-a and -b).

CLSM observation and measurement
After SS-OCT observation, all specimens were fixed in epoxy resin (EpoxiCure, Buehler). After 8 h, a low-speed diamond saw was used to cut each specimen in half along the center and obtain discs with a thickness of approximately 2 mm. Then, the slices were trimmed off using wet 2000-grit silicon carbide papers and further polished with diamond paste down to 0.25 µm under running water. The cross-sectional slice examined using the nondestructive OCT imaging was physically separated and examined under CLSM (1LM21H/W, Lasertec, Yokohama, Japan) at magnifications of 125× to 500×.

The linear measurements of lesion depths were shown in Figs. 2-b and -b'. A baseline was drawn from DEJ to the non-demineralized root surface. The lesion depths of 20 positions were measured with a width of 2,000 µm and an interval of 100 µm. The average was calculated as the value of lesion depth of one specimen. Lesion depths from the baseline to the lesion boundary (LD_{CLSMbaseline}) and that from the lesion surface to the lesion boundary (LD_{CLSM}) were measured.

TMR observation and measurement
After CLSM observation, the 8 specimens with in vitro root caries were processed for TMR analysis as follows: the specimens were cut into sections approximately 200 to 240 µm in thickness using a low-speed diamond saw. TMR images were taken from a central dentin lesion slice using an X-ray generator (SRO-M50, Sofron, Tokyo, Japan) at 25 kV voltage and 4 mA current for 9 min, with a Ni filter. The distance between the X-ray tube and the specimen was 15 cm. The TMR images, together with 15 aluminum step-wedges (each 15 µm in thickness), were captured in the X-ray glass plate film (High Precision Photo Plate PXHW, Konica Minolta Photo, Tokyo, Japan), and scanned as 8-bit digital images using a CCD camera (DP70, Olympus, Tokyo, Japan) attached to a microscope (BX41, Olympus).

Mean mineral profiles (mineral density versus depth) were created using Image J and a custom Visual Basic application written in Microsoft Excel. The mineral density was calculated using the calibration curve, considering that the sound (non-demineralized) contained 48 vol.% mineral density. The lesion depth was defined at a distance from the lesion surface (LD_{TMR}) where the mineral density was 5% less than that in the sound area. The mineral loss (ML) was determined by the integrated mineral loss from the lesion surface to the lesion depth (Figs. 3-c and -d).

Statistical analysis
The normal distributions of LD, SI-distance and ML were checked by Shapiro-Wilk test. Lesion depths of OCT were compared by paired samples t-test. The correlations between lesion depth of OCT and that of CLSM, wet and dry lesion depths of OCT, SI-distance and LD_{TMR}, LD_{TMR} and ML were analyzed by Pearson's correlation test. All the statistical tests were performed at a 0.05 significance level using software (SPSS ver. 16 for windows, Chicago, IL, USA).

RESULTS
Representative OCT images of in vitro root caries are shown in Fig. 4. The thickness of demineralized root dentin increased from Dem1 to Dem2 but reduced from wet to dry conditions. Wet and dry lesions showed distinct differences in the following four parts: lesion surface, lesion body, lesion boundary and sound dentin. In detail, dry lesion surface showed a stronger surface reflection than wet lesion surface. Dry lesion body showed a darker and thinner zone than wet lesion body. Dry lesion boundary showed a clear white line, while...
Fig. 5 Signal intensity (SI) profile of in vitro root caries. Dry lesion had two SI peaks located at the lesion surface (a) and the lesion boundary (c1, c2), while wet lesion had only one SI peak located at lesion surface. Dry lesion surface had a higher SI than wet lesion surface. Dry lesion body (b) had a lower SI than wet lesion body. Wet lesion body had a flat SI and SI started to turn down at the lesion boundary. The sound dentin (d) under dry lesion had higher SI than that under wet lesion. The distance between two SI peaks of Dem2dry (2) was larger than that between two SI peaks of Dem1dry (1). The second SI peak of Dem1dry (c1) was higher than that of Dem2dry (c2). In sound dentin (d), SI was similar between Dem1 and Dem2 under wet or dry conditions.

In vitro root caries under wet and dry conditions show different SI patterns (Fig. 5). Dry lesion had two SI peaks located at lesion surface and lesion boundary, while wet lesion had only one SI peak located at lesion surface. Dry lesion surface had a higher SI than wet lesion surface. Dry lesion body had a lower SI than wet lesion body. Wet lesion body had a flat SI and SI started to turn down at the lesion boundary. The sound dentin under dry conditions had higher SI than that under wet conditions. The distance between the two SI peaks of Dem2dry was larger than that of Dem1dry. The second SI peak of Dem1dry was higher than that of Dem2dry. In sound dentin, SI was similar between Dem1 and Dem2 under wet or dry conditions.

Mean values and standard deviations of SI-distance and lesion depths are shown in Fig. 6-a. Wet lesion depths were significantly higher than dry lesion depths (LD1OCTwet > LD1OCTdry, LD2OCTwet > LD2OCTdry). Lesion depths of Dem2 were significantly higher than that of Dem1 (LD2OCTwet > LD1OCTwet, LD2OCTdry > LD1OCTdry). SI-distance showed a similar value as LD1OCTdry, which was approximately 200 µm. Mineral loss (vol.%) was approximately 39% (Fig. 6-b).

Figure 7 shows correlations between demineralization parameters (OCT and CLSM data, OCT and TMR data) of in vitro root caries. There were significant correlations between lesion depth of OCT and that of CLSM (Figs. 7-a and -b), SI-distance and lesion depth of TMR (LD7m) (Fig. 7-c), LD7m and mineral loss (ML) (Fig. 7-d).

Digital photos, OCT images and signal intensity profiles of three natural root caries are shown in Fig. 8. Under wet conditions, tooth No. 1 showed a lesion...
Fig. 6 Bar graphs with mean value and standard deviations of SI-distance, lesion depths and mineral loss.
(a) Wet lesion depths were significantly higher than dry lesion depths (LD_{OCT, wet} > LD_{OCT, dry}, LD_{2OCT, wet} > LD_{2OCT, dry}). Lesion depths of Dem2 were significantly higher than that of Dem1 (LD_{2OCT, wet} > LD_{1OCT, wet}, LD_{2OCT, dry} > LD_{1OCT, dry}). The straight lines show significant differences between two groups (paired samples t-test, \( p < 0.05 \)). SI-distance had a similar value as LD_{2OCT, dry} (pentagram). (b) The mean value of mineral loss (vol.\%) was approximately 39%.

Fig. 7 Correlations between demineralization parameters of in vitro root caries analyzed by Pearson’s correlation test.
(a) Wet lesion depth of OCT and lesion depth of CLSM with baseline showed a significant correlation (\( p < 0.05 \)). (b) Dry lesion depth of OCT and lesion depth of CLSM without baseline showed a significant correlation (\( p < 0.05 \)). (c) Distance of two SI peaks (SI-distance) and lesion depth of TMR (LD_{TMR}) showed a significant correlation (\( p < 0.05 \)). (d) Lesion depth of TMR (LD_{TMR}) and mineral loss (ML, vol.\%×µm) showed a significant correlation (\( p < 0.05 \)).

boundary. Under dry conditions, teeth No. 1, No. 3 and No. 6 showed lesion boundaries. The demineralized dentin under wet conditions showed bright zones, while that under dry conditions had both bright and dark zones. Two SI peaks existed under dry conditions and the second peak was lower than the first peak.

**DISCUSSION**

In this study, it was verified that the demineralized root dentin shrunk during caries progression and in the process of dehydration as well, which could be monitored by OCT, showing changes in optical properties (Figs. 4,
Fig. 8 OCT images, signal intensity profiles and digital photos of three natural root caries.
Under wet conditions, tooth No. 1 showed a lesion boundary (a solid white triangle).
Under dry conditions, teeth No. 1, No. 3 and No. 6 showed lesion boundaries (blank white triangles). The lesion under wet conditions had bright zones, while the lesions under dry conditions had both bright and dark zones. Signal intensity (SI) profiles showed two SI peaks under dry conditions and the second peak was lower than the first peak.

Previously, some OCT studies have shown that a visual lesion boundary was clearer under hydrated conditions than that under dry conditions\(^{13,16,17}\). However, in this study, the dry lesion boundary had a clear white line but the wet lesion boundary did not on OCT images. Moreover, the dry lesion body was darker than the wet lesion body (Figs. 4 and 8).

In the specimens with \textit{in vitro} root caries, wet lesion boundaries existed between bright zones of demineralized dentin and dark zones of sound dentin without showing white lines on OCT images (Figs. 4-b and -c). On the other hand, lesion boundaries under dry conditions had white lines (Figs. 4-b’ and -c’) with signal peaks (Fig. 5), which was clearer than that under wet conditions. Mineral hydroxyapatite crystals still remained at the lesion boundary, which was between demineralized and sound dentin. The disorganization or denaturation of collagen may trap residual mineral constituents within the hybrid layer after phosphoric acids etching\(^{20}\), which might also happen to the dentin collagen demineralized by bacterial acids. These mineral crystals could cause strong diffused scattering under dry conditions.

Generally, as air inside the porosities of the demineralized dentin increases the diffused scattering within the lesion, dry lesion body looks brighter than wet lesion body. However, in this study, the dry lesion body showed as a dark zone, which indicated OCT images of lesions might be also related to microstructural changes in demineralized dentin from wetting to drying. Signal fluctuations are associated not only with changes in the porosity, but also in structural phase (organic versus mineral-organic) and optical properties in the lesion body\(^{21,22}\).

There was a volume shrinkage of collagen due to complete drying and the density of these remaining mineral crystals increased. Under dry conditions, collagen fibrils move close together resulting in passive collapse of the soft collagen network\(^{23}\). The possible explanations of the shrinkage and collapse of the demineralized collagen network after water loss include the surface tension force acting on air-collagen fibrils interface\(^{24}\) and the shortening of the interconnected collagen fibrils\(^{25}\). Since collagen is optically nonlinear and is known to scatter light, gelatinized or fragmented collagen appears less scattering\(^{26}\). Then if the porosities inside the dry lesion reduce, the density of collage will increase and light waves will slow down upon when entering a denser medium. Also the mineral-deprived dentin matrix may appear as a dark zone under OCT due to the loss of main scatters\(^{27}\). Moreover the directions of demineralized dentinal tubules affected OCT images\(^{28}\). These possible factors resulted in a backscattering reduction in dry lesion body, which showed darker SS-OCT images with reduced signal intensities than wet lesion body. Similar phenomenon was observed in a study using PS-OCT\(^{29}\). In that study, the shrinkage and lack of optical changes in cementum as a result of demineralization were found and the cementum layer was clearly visible as a transparent zone above the highly scattering dentin layer in lesion areas. In this study, we demonstrated that a conventional OCT without polarization sensitivity could be used to compare optical properties of root caries under different conditions, providing both non-destructive cross-sectional images and signal intensity profiles to make the study more understandable and easier to analyze than previous
studies.

The thickness of dry lesion body was thinner than that of wet lesion body on OCT images (Fig. 4). Also, dry lesion depth was significantly lower than wet lesion depth (Fig. 6-a). When demineralized dentin dried, it shrank, got twisted and buckled. Demineralized dentin underwent 18% linear shrinkage during air exposure and under vacuum in specimen preparation for scanning electron microscopy. Also, demineralized dentin underwent an approximately 65% volumetric shrinkage when allowed to air-dry at 37°C and 28% relative humidity.

Because the distance between two SI peaks showed almost the same value of dry lesion depth of OCT (Fig. 6-a), the position of the second SI peak was the lesion boundary on OCT images (Fig. 5). The second SI peak located where mineral loss was 39% on TMR images (Fig. 6-b). Moreover, the distance between two SI peaks after Dem2 of in vitro dry lesion (SI-distance) and mineral loss (ML) of lesion body correlated well (Fig. 7-c). These results implied that the more mineral loss, the more shrinkage of the lesion in the process of dehydration. The implication of this SS-OCT study was confirmed in previous PS-OCT studies, which showed that the degree of shrinkage correlates with mineral loss and that the reflectivity loss correlated mineral loss. It was further demonstrated that if the lesions are exposed to a remineralization solution they no longer shrink. However, in those PS-OCT studies, extensive shrinkage was found in PS-OCT and TMR images compared to polarized light microscopy (PLM) images rather than using the same non-destructive technique —OCT only. Our study not only showed the shrinkage of dentin lesions during caries progression and in the process of dehydration, but also showed the depth-profile of dB signal intensity and distinct differences in the lesion boundary under wet and dry conditions, using SS-OCT only.

The degree of shrinkage correlated with mineral loss, which might be resulted from the porosities increase in dentin collagen and the gelatinization of dentin collagen after demineralization. The caries process in dentin can be divided into two distinct steps: acid dissolution of the mineralized phase of the tooth and degradation of the dentin collagen matrix by proteolytic enzyme action. The demineralization has been demonstrated to be caused by bacterial acids. The exposed dentin matrix, containing type I collagen and non-collagenous proteins, is degraded by host collagenolytic enzymes, MMPs and cysteine cathepsins (CCs). Furthermore, during caries progression, there is an increase in micro- and nano-porosities due to changes in dentin collagen structure and distribution and noncollagenous protein, synergistically contributing to reductions in physical and mechanical dentin properties. Demineralized dentin has two types of porosity: the opened tubules and micro-branchings and the inter-fibrillar spaces. Also, caries-infected dentin showed a complete denaturation of the dentin collagen network into a gelatinized mass of micro-fibrils. Transition zone showed a generalized retention of collagen fibrillar structure, despite the absence of cross-banding from individual collagen fibrils. Derangement of some collagen fibrils into microfibrillar stands could also be recognized.

In this study, 3 out of 6 natural root caries under dry conditions showed similar OCT images and SI patterns as in vitro root caries (Fig. 8). Dry lesion body showed dark zones with two SI peaks located at the lesion surface and boundary. However, not all natural root caries showed like this. In vitro or artificial caries have similar homogeneous caries patterns, while natural caries has inhomogeneous and complicated microstructural patterns, which reflect the pathomorphological reaction of dentin to caries attack in each tooth and the consequence of demineralization and remineralization during caries progress. However, the bacterial demineralization by forming the base of the biofilm in OBR appears to be a more clinically relevant approach over the conventional demineralization using acidic solutions and the lesion induced by cariogenic bacteria has not been shown before in similar kind of studies.

In order to provide a stable dry condition and to prevent strong air blow destroying the lesion surfaces of all the specimens tested in this study, the specimens were allowed to dry naturally through water evaporation at room temperature for 1 h. Further development of the methodology will include controlled drying using an air syringe with several seconds that allows reproducibility of the drying process in clinic.

In short, the first null hypothesis of this study was rejected as in vitro root caries under wet and dry conditions showed different OCT images and optical properties. The second null hypothesis was partially rejected as some but not all natural root caries showed similar OCT images and optical properties as in vitro root caries.

CONCLUSION

Within the limitation of this study, SS-OCT can be used to monitor dentin lesion shrinkage during caries progression and in the process of dehydration. The base of demineralized dentin can be detected by SS-OCT more clearly under dry conditions than under wet conditions.

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