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Root dentin body moisture changes caused by ascorbic acid

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Abstract

Objectives—To quantify *in vitro* the root dentin moisture (DM) when soaked in 10% ascorbic acid solution (Group A) and distilled water (Group B) for up to 14 days.

Methods—Forty-four extracted human mandibular single-rooted teeth were sectioned perpendicular to the long axis at the CEJ to access the root dentin. The samples were divided into groups A and B. Baseline (day 0) DM was measured using a digital grain moisture meter. One sample was placed in each tube, which was then filled with solution. All tubes were kept at 37°C and 100% humidity. DM was measured after 1, 3, 7, and 14 days. The baseline DM value was used as a covariate in the generalized estimating equation (GEE) analysis to account for the difference in the baseline DM between the two groups.

Results—The mean DM(%) values $\pm$ standard deviation on days 0, 1, 3, 7, and 14 were 11.4 ± 1.08 , 17.1 ± 0.87 , 18.2 ± 0.76 , 18.4 ± 0.77 , and 17.9 ± 0.90 in Group A, and 10.2 ± 0.95 , 12.8 ± 0.90 , 13.3 ± 0.95 , 13.0 ± 0.91 , and 13.2 ± 0.89 in Group B, respectively. Group A had significantly higher baseline DM than Group B ($p=0.006$). After adjusting for differences in the baseline, the GEE analysis indicated that, on average, Group A had a significantly higher increase in DM than Group B, with means $\pm$ standard deviation of 4.35 ± 0.26 .

Conclusions—The moisture increase in the ascorbic acid group was greater than that in the distilled water group. Soaking root dentin in the unreplenished ascorbic acid solution or distilled water beyond 3 days did not further increase DM.

Keywords

Ascorbic acid; Bonding; Dentin moisture; Generalized estimating equation analysis; Rapid non-destructive method

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Introduction

L-ascorbic acid (AA) is the white to light-yellow, water-soluble vitamin (1) commonly known as vitamin C. Sodium ascorbate (SA) is a salt of AA. Both AA and SA have antioxidant properties. A 10 wt % AA solution possesses an acidity of approximately pH 2 (1,2), whereas SA is a neutral biocompatible antioxidant (3). An AA solution gradually oxidizes and becomes less reductive (2).

It is known that AA enhances the dentin bonding strength of adhesive resins when used on dentin surfaces as an experimental conditioner for C&B Metabond (1). SA is known to enhance the bonding strength of adhesive resins on dentin surfaces treated with sodium hypochlorite (NaOCl) in endodontic and operative dentistry (4-8). Morris et al. reported that there was no statistical difference between AA and SA in terms of their effect on bonding strength (5).

Effective bonding strength is thought to occur when dentin collagen changes from an oxidized substrate to a reduced substrate, which restores its redox potential. Regarding dentin bonding strength in general, it is known that an optimally moist dentin surface improves the bond strength of an adhesive system; thus, wet bonding techniques are employed in the clinical setting (9,10). Dentin surface moisture can also be influenced by dentin body moisture, which varies significantly in reference to different tooth conditions. While it is known that AA and SA promote bonding strength, it is not known whether dentin body moisture changes are caused by AA and SA.

Recently, dentin bonding research has expanded to the field of endodontics because long-term endodontic treatment success is believed to depend on the prevention of apical and coronal leakage (11-14). To enhance the hydraulic seal, the resin-based adhesive bonding obturation system (15-18) and post/core system (19-21) have been developed for use in clinical endodontics.

However, root dentin body moisture reports are scarce (22), perhaps because the traditional dentin moisture measurement technique involves desiccation of samples to calculate weight change. This technique is time-consuming and requires irreversible sample destruction. In contrast, a novel technique using a moisture content meter that accurately records the true dentin moisture content (22) was used in the present study. Briefly, in 1990, a nondestructive method to estimate the humidity content of a single peanut kernel was accomplished by holding the kernel between two plates and measuring the moisture in it using a parallel plate capacitor (23). This digital grain moisture meter (TA-5, OGA Electric, Tochigi, Japan) is a commercial machine using impedance methodology; a diagram of its internal structure is shown in Figure 1a. This nondestructive grain moisture testing machine was calibrated using samples with known moisture content to measure the impedance of each sample (24). Although grains and teeth are structurally different, the previous study indicated that a moisture measuring mechanism utilizing impedance technology can be successfully used in dentistry (22). The statistical analysis showed that the five grain modes of the digital grain moisture meter had high validity, which generally indicated that they can be used for dentin moisture measurements.

The aim of this study was to examine chronological root dentin moisture changes caused by ascorbic acid (AA) on extracted human teeth.

Materials and Methods

The digital grain moisture meter (TA-5, OGA Electric, Tochigi, Japan) employed in this study was originally designed to measure the moisture in six different types of grain: unpolished rice, unhulled rice, polished rice, barley, wheat, and rye. The accuracy (detection level) of the

machine's readings for these grains is 0.1%. In the pilot study (22), the results indicated that the digital grain moisture meter accurately recorded the true dentin moisture content except for the polished rice mode. All other modes were acceptable for recording the true dentin moisture content. Therefore, in this study, the wheat mode was used for the measurements.

Forty-four extracted human mandibular single-rooted teeth were placed in distilled water after extraction. The teeth were washed in running tap water overnight, were sectioned perpendicular to the long axis at the CEJ with a diamond saw (Buehler, Lake Bluff, IL), and the coronal parts were discarded in order to access the root dentin. The root surface was cleaned, scaled, and planed for cementum removal. The pulp was mechanically removed by hand files without irrigants. All the samples were put into laboratory dishes and equilibrated in the tissue culture incubator (Nu Aire, Plymouth, MN) for 48 hours at 37°C and 100% humidity. The moisture in each dentin sample was measured five times using the grain moisture meter at the wheat mode setting. To avoid moisture evaporation, the samples were held in sealed plastic bags until the measurements were taken. The completion of this procedure was defined as the baseline, day 0 value. The step-by-step measurement method is shown in Figure 1b.

The 44 samples were divided into two groups of 22 samples each. Those in Group A were immersed in 10 wt% L-Ascorbic acid solution (Product Number A5960, Lot Number 065K0181; Sigma-Aldrich, St. Louis, MO), while the samples in Group B were immersed in distilled water, and served as the control. One sample was placed in each polyethylene centrifuge tube (Fisher Scientific, Pittsburgh, PA), and the solution or distilled water was added to the tube to completely submerge the sample. All the tubes were kept at 37°C and 100% humidity. The dentin moisture was measured after 1, 3, 7, and 14 days. At each measurement, the polyethylene centrifuge tube cap was opened, and the root dentin sample was removed with cotton pliers. The excess solution on the sample was gently wiped off using a Kimwipe before the sample was measured. The moisture in each dentin sample was immediately measured five times using the grain moisture meter at the wheat mode setting. Upon completion of the measurements, the sample was returned to the polyethylene centrifuge tube. The solution was not changed, and the sample was completely submerged again. The polyethylene centrifuge tube was capped and kept at 37°C and 100% humidity until the next scheduled measurement.

Statistical Analysis

The moisture meter measurements taken on days 0, 1, 3, 7, and 14 from all 44 samples are expressed as mean $\pm$ standard deviation (SD) for each group (Group A: ascorbic acid and Group B: distilled water as control). The dentin moisture at baseline (day 0) for groups A and B was compared using a two-sample t-test at the 5% level of significance.

The generalized estimating equation (GEE) approach was employed to investigate whether groups A and B exhibited significant differences in dentin moisture over time, accounting for the correlation among the repeated measurements. The model includes dentin moisture at baseline as a covariate to control for the baseline differences. The GEE method has been shown to be robust against misspecification of the true correlation structure (25).

The minimal required period for soaking root dentin in different solutions to reach the highest peak was identified by paired t-tests among different time points within each group. All computations were carried out with the SAS v.9.1.3. statistical package (SAS Institute, Cary, NC).

Results

The mean dentin moisture (%) and standard deviation (SD) on days 0, 1, 3, 7, and 14 were 11.4 ± 1.08 , 17.1 ± 0.87 , 18.2 ± 0.76 , 18.4 ± 0.77 , and 17.9 ± 0.90 in group A, 10.2 ± 0.95 , 12.8 ± 0.90 , 13.3

± 0.95 , 13.0 ± 0.91 , and 13.2 ± 0.89 in group B, respectively. (Table 1) Group A had significantly higher baseline dentin moisture than group B ($p=0.006$).

The GEE analysis suggests that, after adjusting for the baseline difference, group A had significantly higher dentin moisture on average (mean 4.35 and standard deviation 0.26) than group B.

The pair-wise comparisons among different time points within each group indicate that soaking root dentin in the AA solution or distilled water up to 3 days significantly increased dentin moisture, but there was no further significant increase afterward.

Discussion

While it is known that L-ascorbic acid (AA) and sodium ascorbate (SA) enhance dentin bonding strength, it is not known whether dentin body moisture changes are caused by AA and SA. Since Morris et al. reported that there was no statistical difference between AA and SA in their effect on bonding strength (5), AA was used in this *in vitro* study. The average dentin moisture in the AA group from day 0 to day 14 significantly increased compared with that in the control group ($p<0.001$). The estimated increased mean (SD) was 4.35 (0.26). AA significantly increased the dentin moisture within the AA group. However, soaking the root dentin in AA solution for more than 3 days did not further increase the dentin moisture.

In order to glean as much information as possible from this study, the pH and color changes of the water and AA solution were also carefully observed in selected samples (#1, #11, and #21). The pH of the observed water samples was 7.0 on day 1 and 7.0 on day 14. The pH of the AA samples was 2.0 on day 1 and 4.5 on day 14. Thus, the pH of the water remained neutral throughout the study, while the pH of AA had increased by the end of the study. It is known that a 10 wt% AA solution possesses an acidity of approximately pH 2 (1,2). Thus, it is likely that a chemical change occurred in the AA group during the course of this study. A clearly observable phenomenon that supports this speculation is the incremental change in the color of the AA solution. On day 1, the solution was clear, while, on day 3, it was bright yellow. By day 7, the color had turned dark yellow, and by day 14, it was very dark yellow. These observations of incremental pH and color changes found in this study correspond to those previously noted when AA solutions gradually oxidize and become less reductive (2). If the AA solution is frequently (e.g., every 24 hours) replaced by freshly prepared solution, a different result might be obtained. Due to the clinical implications of dentin bonding procedures, it is critical to confirm the pH and color of the commercially available stock AA solution, in case the solution has altered during storage.

An increase in humidity is believed to occur when the dentin collagen changes. AA might swell the collagen in dentin and, therefore, the dentin would retain water. In a clinical application, the duration of time in which AA is applied only to the dentin surface to increase dentin bonding strength is less than one minute, and thus its penetration depth may be limited. Since there is no current technology to quantitatively assess the dentin surface moisture, the current study examined root dentin body moisture for up to 14 days, which is longer and more robust than in a clinical setting. The highest level of moisture content in dentin body was considered by expecting the maximum influence to dentin collagen. While a wet dentin bonding technique is recommended, significantly altered collagen in which the dentin retains excessive water is probably not desirable for successful bonding. Future studies on tensile bonding will test different degrees of dentin moisture using this new, non-destructive, quantitative method, which is expected to answer more definitively the clinical questions surrounding step-by-step dentin bonding techniques that currently depend heavily on the senses and experiences of individual dentists.

Although little is known about the effect of AA solution on the extracted dentin and its components, Franceschi et al. reported that AA is a molecule that stimulates matrix production, mainly by increasing the amount of type I collagen (26-28). Further, AA is of interest to those in emerging areas of dental pulp stem cell research, particularly in reference to the influence of dentin collagen on matrix production. Balic et al. reported that AA is added to induce the differentiation of dental pulp (29). A future direction of AA research should not only be the establishment of a standard dentin bonding technique, but also the enhancement of the treatment modality.

Conclusion

1. The average dentin moisture in the ascorbic acid group over the time period tested significantly increased compared to that in the control group ($p < 0.001$). The estimated increased mean (SD) was 4.35 (0.26).
2. L-ascorbic acid significantly increased the dentin moisture within the ascorbic acid group over time. Soaking the root dentin in unreplenished ascorbic acid solution longer than 3 days did not further increase the dentin moisture.

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Figure 1a **Figure 1b** **Figure 1.**

Figure 1a

A map of the internal structure of the grain moisture testing machine (TA-5, OGA Electric, Tochigi, Japan)

C; capacitor plates, I; impedance measurement

Figure 1b

The step by step measurement method of the grain moisture testing machine

1. The picture of the machine,
2. The root dentin sample was placed on the moisture measuring plate.
3. The moisture measuring plate was inserted into the machine main body.
4. The screw chamber was tightened by hand to hold the moisture measuring plate and the root sample.
5. The button was pressed to measure the moisture.
6. The moisture was digitally displayed.

Table 1
Average dentin moisture over time