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Chronological comparison of root dentin moisture in extracted human teeth stored in formalin, sodium azide, and distilled water

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Abstract

This study quantified *in vitro* the root dentin moisture when 10% formalin (Group A), 3% sodium azide (Group B), and distilled water (Group C) were used as teeth storage media. The root dentin moisture of 66 extracted human mandibular single-rooted teeth was measured at baseline (day 0) and at 1, 3, 7, and 14 days using a digital grain moisture meter. The baseline dentin moisture value was used as a covariate in the generalized estimating equation (GEE) analysis. The mean dentin moisture values (%) $\pm$ standard deviation on days 0, 1, 3, 7, and 14 were 10.6 $\pm$ 0.64, 14.3 $\pm$ 0.71, 14.6 $\pm$ 0.84, 14.4 $\pm$ 0.64, and 14.7 $\pm$ 0.75 (Group A); 11.4 $\pm$ 0.94, 14.6 $\pm$ 0.95, 14.6 $\pm$ 0.76, 14.6 $\pm$ 0.93, and 14.8 $\pm$ 0.81 (Group B); and 10.2 $\pm$ 0.95, 12.8 $\pm$ 0.90, 13.3 $\pm$ 0.95, 13.0 $\pm$ 0.91, and 13.2 $\pm$ 0.89 (Group C), respectively. The dentin moisture increased in all three groups; however, there was no overall significant difference in moisture between the formalin and sodium azide groups.

Keywords

Dentin moisture; Rapid non-destructive method; Impedance; Dentin-material interface; Structural strength of dentin; Formalin; Sodium azide; Bonding; Storage media

Introduction

Extracted human teeth are widely used for *in vitro* bond strength studies of adhesive restorative materials to dentin. A variety of media and methods have been used to store the teeth, keep them moist, and kill the bacteria in the teeth¹. The teeth are stored, for example, in neutral buffered formalin¹⁻³ or sodium azide^{4, 5} until their use in a research laboratory.

An optimally moist dentin surface improves bond strengths when using adhesive systems and wet bonding techniques^{6, 7}. Ozok *et al.* reported that a significant source of dentin moisture during clinical use of adhesive techniques in vital teeth is dentinal fluid, which is modeled by

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intrapulpal pressure⁸. However, dentin moisture in extracted human teeth lacks dentinal fluid. Thus, *in vitro*, dentin surface moisture can be influenced by dentin body moisture, which varies significantly in different tooth conditions. While the importance of dentin moisture in dentin bonding research is known, the effect of the storage media prior to their use with respect to dentin body moisture changes has not been characterized.

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 leakage⁹⁻¹². To enhance the hydraulic seal, the resin-based adhesive bonding obturation system¹³⁻¹⁶ and the post/core system¹⁷⁻¹⁹ have been developed for use in clinical endodontics. However, reports on root dentin body moisture are scarce²⁰.

One reason for this shortage of reports may be that the traditional dentin moisture measurement technique involves the desiccation of samples to calculate weight change, a technique that 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 content²⁰ was used in this 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 capacitor²¹. The digital grain moisture meter (TA-5, OGA Electric, Tochigi, Japan) is a commercial machine using the impedance methodology. This nondestructive grain moisture testing machine was calibrated using samples with known moisture content in order to measure the impedance of each sample²². Although grains and teeth are structurally different, the previous study indicated that a moisture measuring mechanism using impedance can be successfully used in dentistry²⁰. The statistical analysis showed that the five grain modes of the digital grain moisture meter had high validity, which was a general indicator that they can be used for dentin moisture measurements.

The aim of this study was to chronologically compare the root dentin moisture of teeth stored in formalin, sodium azide, and distilled water.

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 of the machine's readings for these grains is 0.1%. In a pilot study²⁰, 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 accepted as recording the true dentin moisture content. Therefore, in the present study, the wheat mode was used for the measurements.

Sixty-six extracted human mandibular single-rooted teeth were placed in distilled water after extraction. The teeth were washed in running tap water overnight, sectioned perpendicular to the long axis at the CEJ with a diamond saw (Buehler, Lake Bluff, IL), and the coronal parts were discarded 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 a laboratory dish and equilibrated in the tissue culture incubator (Nu Aire, Plymouth, MN) for 48 hours at 37°C and 100% humidity. The moisture of each dentin sample was measured five times using the grain meter at the wheat mode setting. The samples were kept in a sealed plastic bag to avoid moisture evaporation until measurements were taken. The completion of this procedure was defined as the baseline value for day 0. The step-by-step measurement method is shown in Figure 1.

The 66 samples were randomly divided into three groups of 22 samples each. Those in Group A were immersed in 10 wt% neutral buffered formalin solution (Product Number HT50-1

–128; Sigma-Aldrich, St. Louis, MO), while the samples in Group B were immersed in 0.3 wt % sodium azide solution (Product Number S2002; Sigma-Aldrich, St. Louis, MO). Distilled water was used for the control samples in Group C. Each sample was placed in a polyethylene centrifuge tube (Fisher Scientific, Pittsburgh, PA), and solution was added to fill the tube and 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 prior to the time it was measured. The polyethylene centrifuge tube was capped, and the tubes were kept at 37°C and 100% humidity until the next scheduled measurement.

Statistical Methods

The moisture meter measurements taken on days 0, 1, 3, 7, and 14 from all 66 samples are expressed as mean $\pm$ standard deviation (SD) for each group (Group A: formalin; Group B: sodium azide) and compared with the control group (Group C; distilled water). A one-way analysis of variance (ANOVA) was conducted to investigate if the dentin moisture at baseline (day 0) was significantly different among the three groups. If significance was found for the omnibus ANOVA test, a Newman-Keuls multiple comparisons test was used to perform pairwise comparisons. Statistical decisions were made at $p=0.05$, unless otherwise stated.

The generalized estimating equation (GEE) has been widely used in the analysis of repeated measurement data due to its robustness at identifying the misspecification of the true correlation structure²³. The GEE approach was used to investigate whether the dentin moisture was significantly different among the three groups over time. The baseline (day 0) dentin moisture value was used as a covariate in the GEE analysis to account for the difference in baseline dentin moisture among the three groups.

The minimal required period for soaking root dentin in different solutions to reach the highest dentin moisture was assessed by paired t-tests among different time points within each group. All analyses were performed using 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 10.6 ± 0.64 , 14.3 ± 0.71 , 14.6 ± 0.84 , 14.4 ± 0.64 , and 14.7 ± 0.75 in group A, 11.4 ± 0.94 , 14.6 ± 0.95 , 14.6 ± 0.76 , 14.6 ± 0.93 , and 14.8 ± 0.81 in group B, 10.2 ± 0.95 , 12.8 ± 0.90 , 13.3 ± 0.95 , 13.0 ± 0.91 , and 13.2 ± 0.89 in group C, respectively. (Figure 2) The ANOVA analysis showed that dentin moisture at day 0 was significantly different among the three groups ($p<0.001$). The Newman-Keuls multiple comparison test indicated that the group A and C were not significantly different in terms of baseline (day 0) dentin moisture, but both groups were significantly different from the group B.

After adjusting for the differences in baseline (day 0) dentin moisture, GEE analysis indicated that, on average, the group A and B, with means (SD) 1.32 (0.19) and 1.22 (0.25), respectively, had significantly higher dentin moisture than the group C. There was no overall significant difference in dentin moisture between groups A and B. Furthermore, there was a significant time (days) effect with day 1 significantly lower than the other days.

The pair-wise comparisons among different time points within each group indicate that soaking root dentin in group A solution for 1 day significantly increases dentin moisture (p-value<0.001). However, soaking the root dentin in group A for 3 days or more does not further increase the moisture. The same conclusion applies to the group B. For the control group C, it suggests that soaking the dentin root in water for up to 3 days significantly increases moisture, but no significant increase afterward.

Discussion

Formalin is composed of formaldehyde, methyl alcohol, and sodium acetate in water²⁴. Formaldehyde acts to preserve tissues by causing the cross-linking of proteins, glycoproteins, nucleic acids, and polysaccharides, which form insoluble methylene bridge products. The cross-linking of these macromolecules fixes the specimen and prevents the degradation of tissues after cell death occurs²⁵. Thus, neutral-buffered formalin has been used routinely for the preservation of pathological specimens, which allows long-term storage². The infection control guidelines from the Centers for Disease Control and Prevention recommend that extracted teeth used for educational or research purposes be stored in 10% formalin for two weeks²⁶⁻²⁸. Other techniques, for example, gamma-ray sterilization of extracted teeth and storage in distilled water are also used²⁹.

It is known that a range of biomechanical features such as the collagen cross-link content of dentin is affected by moisture³⁰; however, the precise distribution and arrangement of water molecules within the collagen-hydroxyapatite of human dentin are not known. It has been calculated that 75.2% of the water is in the tubules and 24.8% of the water is in the mineralized matrix³¹. In the results of the present study, after adjusting for the differences in baseline dentin moisture, the generalized linear model indicated that, on average, the formalin group had significantly higher dentin moisture compared to the water group with means (SD) of 1.32 (0.19). Soaking root dentin in formalin beyond 3 days does not further increase the dentin moisture.

Retief *et al.* stored teeth for 2 days or for 6 months in buffered formalin before testing the shear bond strengths of resin to dentin³². Their results showed that the differences between 2 days and 6 months were not statistically significant. If the shear bond strength correlates with dentin moisture, the fact that soaking root dentin in formalin beyond 3 days does not further increase dentin moisture may be partially explained. Further, Titley *et al.* found little effect of formalin on the bond strength to bovine dentin¹. The current study indicated that dentin moisture increased in the first few days. It is speculated that the cross-linkage of collagen may occur in the first few days.

Sodium azide is a commonly used preservative for laboratory reagents. As a storage medium, sodium azide has been used to inhibit microbial growth in teeth due to a mechanism involving metal ion complexation and displacement from enzymes. Sodium azide is not a fixative; thus, the cross-linking of collagen is less expected. However, with respect to dentin moisture, there is no overall significant difference in dentin moisture between the formalin and sodium azide groups. As with formalin, dentin moisture in sodium azide increased in the first few days and then stabilized.

Distilled water was used as a control in the present study. Addy and Mostafa used distilled water as a tooth storage medium³³. Although distilled water will not change the dentin properties such as the cross-linkage of collagen, special attention should be paid to infection control. In future research, if the issue of infection control has been addressed, formalin and sodium azide could be used immediately after the tooth extraction as a storage medium.

Jameson *et al.* reported on whether storage media and time cause any changes in water loss through the dehydration of the dentin². While they used the traditional method of determining weight change and tracing water loss to measure moisture, the current study used a novel, rapid, non-destructive method²⁰. This new method is suitable for studying the impact of dentin moisture content on teeth during *in vitro* bonding experimentation. It is possible in future research to combine this technique with the *in vitro* dentin bonding experimentation. To quantitatively determine the optimal level of dentin moisture with the highest dentin bonding strength may provide us with useful information that will lead to the next phase of research with the goal of using impedance for rapid and non-destructive dentin moisture measurement in the clinical setting.

Conclusions

Soaking root dentin in formalin or sodium azide solution beyond 1 day, or soaking root dentin in water beyond 3 days, does not further increase dentin moisture.

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Figure 1. 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.

Average dentin moisture over time

Figure 2.
Average dentin moisture over time