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Qiang Zhu

University of Connecticut School of Medicine and Dentistry

Jin Jiang

University of Connecticut School of Medicine and Dentistry

Kamran E. Safavi

University of Connecticut School of Medicine and Dentistry

Larz S.W. Spångberg

University of Connecticut School of Medicine and Dentistry

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A rapid non-destructive method for root dentin moisture measurements:

In vitro pilot study

Takashi Komabayashi, DDS, M. Dent. Sc., PhD*, Qiang Zhu, DDS, PhD**, Jin Jiang, DDS, PhD**, Kamran E. Safavi, DMD, M.Ed.**, and Larz S.W. Spångberg, DDS, PhD**

*Department of Endodontics, Texas A&M Health Science Center Baylor College of Dentistry

**Division of Endodontology, Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut

Abstract

Dentin moisture content is important in adhesive bonding and structural strength research. However, there is no rapid method available to assess dentin moisture without sample destruction. This study examined the use of a digital grain moisture meter to measure root dentin moisture *in vitro*. Extracted mandibular single rooted teeth were sectioned at the CEJ. The moisture of the root dentin was measured at six measuring modes for different grains and repeated five times. Dentin weight changes before and after drying were measured to obtain control values. The control values were compared with machine readings. In conclusion, (1) Each non-destructive measurement took less than 30 seconds. (2) 24 hours storage at 37°C and 100 % humidity did not restore dentin moisture. (3) Five grain modes had a high validity, and could be used for dentin moisture measurements.

Keywords

Dentin moisture; Rapid non-destructive method; Digital grain moisture meter; Impedance; Dentin-material interface; Structural strength of dentin

Introduction

Long term endodontic treatment success is in some degree dependent on the prevention of apical and coronal leakage 1-4. To enhance the hydraulic seal of root canals, resin based adhesive bonding obturation system 5-8 and post/core system 9-11 have been developed for clinical endodontics. Moisture control is exercised prior to using these resin based materials.

Name and address for correspondence, including fax number, telephone number and e-mail address; Takashi Komabayashi DDS, M. Dent. Sc., PhD, Assistant Professor, Department of Endodontics, Texas A&M Health Science Center Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246 USA, Tel: 214-828-8365, Fax: 214-874-4507, E-mail addresses: tkomabayashi@bcd.tamhsc.edu, ICD38719@nifty.com.

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Research indicates that dentin moisture content affects both the outcome of sealing dentin-material interface and the structural strength of the treated tooth 12-15. Moisture content of dentin is critical for the integrity of the intertubular collagen network and dentin tubules 16, 17. Excessively high moisture content of dentin or residual liquid in the root canal system can prevent sealing of the dentin-material interface, whereas excessive desiccation may collapse the demineralized dentin and compromise resin-based sealer infiltration. Accordingly, an optimally moist dentin surface improves bond strengths when using adhesive system and wet bonding techniques 18, 19.

In the operative dentistry setting, the dentin surface moisture is clinically controlled by the gentle use of compressed air 20. However there is no consensus regarding the proper surface moisture content 21. Dentin moisture is described in vague terms, for example, “no dump”, “slight surface shines”, and “not bone dry”. These terms only describe the surface wetness and have no objective meaning. In current practice, proper control of dentin moisture is subjective and technique-sensitive due to lack of an objective dentin moisture measurement device and communication methodology.

For example, dentin body moisture content may be high in the tooth of a young individual, whereas it may be low in an endodontically treated tooth, which may become more susceptible to fracture than vital teeth 22, 23. It has also been proposed that loss of pulp vitality alters the moisture content of dentin 24-27. There is presently no definitive proof of any association between mechanical weakening of dentin and moisture content, and considerations of dentin body moisture have been given limited attention.

When studying dentin strength and various applications of dentin bonding in the laboratory the control of dentin moisture becomes an even more important factor as the tooth is no longer in the moisture controlling environment of the mouth and attached to living tissues. However, there is no accurate measuring device for rapid nondestructive determination of moisture of dentin body and surface. Traditional dentin moisture measurement techniques involve desiccation of samples to calculate weight change. This technique is time consuming and requires irreversible sample destruction.

In the agricultural industry, scientific devices are used to measure the content of water in grains. Grain moisture measuring instruments were developed after Briggs discovered the existence of linear relationship between the logarithm of the electrical resistance of wheat and its moisture content in 1977 28. In 1990, a nondestructive method to estimate the humidity content of single peanut kernel was accomplished by measurements on a small parallel-plate capacitor holding the kernel between the plates 29. A nondestructive grain moisture testing machine is calibrated using samples with known moisture content to associate the impedance of each sample 30. The digital grain moisture meter (TA-5, OGA Electric, Japan) is a commercial machine using the impedance methodology and its map of the internal structure is shown in Figure 1a.

Grains and teeth are structurally different. In endodontics, Sunada reported the correlation between root dentin and impedance, which later became the fundamental mechanism of the electric apex locator 31, 32. In operative dentistry, Prepometer (Hager Worldwide, Odessa,

FL) measures the thickness of dentin above the pulp during crown preparation by using the mechanism of impedance 33. The use of the mechanism of impedance to develop these instruments provided the authors with the idea to investigate the use of a commercial digital grain moisture meter for root dentin moisture measurement, which would be both rapid and non-destructive. The objective of this study is to ascertain if a commercial digital grain moisture meter can be reliably applied for rapid measurements of dentin body moisture measurement *in vitro*.

Materials and Methods

The experiment was conducted in four parts:

Part 1. Establish the consistency of record reading when using a digital grain moisture meter for dentin measurements

A digital grain moisture meter (TA-5, OGA Electric, Japan) was originally designed to measure moisture for six different grains: *unpolished rice*, *unhulled rice*, *polished rice*, *barley*, *wheat*, and *rye*. The accuracy of machine reading is 0.1 % for these grains. However, it is unknown whether this method is suitable for root dentin moisture measurement. Therefore, the consistency of the machine readings was examined by root dentin specimens as follows.

Eight extracted human mandibular single-rooted teeth were sectioned with a diamond saw (Buehler-Lake Bluff, IL) perpendicularly to the long axis at the CEJ, and coronal parts were discarded to obtain root dentin. The root surface was cleaned, scaled, and planed for cementum removal. The pulp was mechanically removed by hand files without irrigation.

The moisture of the root dentin was measured by the digital grain moisture meter at all six grain modes and repeated five times in each mode. The step by step measurement method is shown in Figure 1b. The data was statistically analyzed using the coefficient of variation, which was calculated as standard deviation /mean.

Part 2. Determine the time required to complete desiccation of dentin

Traditional dentin moisture measurement techniques involve desiccation of samples in order to calculate weight change. Accordingly, the eight specimens used in Part 1 were used to determine the time required to complete desiccation of dentin.

First, the eight specimens of root dentin were equilibrated in a tissue culture incubator for 48 hours at 37 °C and 100 % humidity (Nu Aire, MN). Second, sample weights were immediately measured to an accuracy of 100 µg (Acculab, LA-60, Germany). Third, samples were placed in a micro hybridization oven at 100 °C (Bellco Glass Inc, NJ). Finally, sample weights were immediately measured and recorded every hour for 8 hours, expecting to obtain 0% humidity. The data showing the rate of decrease were plotted on a graph.

Part 3. Co-relationship analysis of 6 measuring modes in digital grain moisture meter before and after desiccation of dentin

We examined which modes of the digital grain moisture meter can be used for dentin moisture measurement. Digital grain moisture meter readings in six modes were compared to the true moisture content, which used the traditional method of weight changes before and after desiccation.

Seventeen freshly extracted human mandibular single-rooted teeth were used to prepare seventeen specimens of root dentin by using the method described in Part 1.

This study part was accomplished in steps. First, samples were put into a laboratory dish and equilibrated in the tissue culture incubator for 48 hours at 37 °C and 100 % humidity. Thereafter, the moisture of dentin samples was measured by the grain meter at all six grain modes and repeated five times for each sample. Immediately after grain meter reading, the samples were weighed to obtain the before desiccation weight. During this time period (approximately 1 hour) the samples were allow to air dry before being returned to 100% humid environment. After 24 hours a second reading with the grain meter were conducted in conjunction with weight measurement. This second reading was defined as time 24 hours. All samples were desiccated to 0% humidity in an oven for 8 hours at 100 °C. Weight measurement was conducted to obtain after desiccation value. The difference between before and after desiccation weight value was calculated.

Part 4; Statistical analysis

Average and standard deviation of 17 samples in 6 measuring modes, which were displayed on the grain machine were recorded.

The true moisture content was calculated after comparing the weight before and after desiccation. The change can be recalculated as the true moisture content of each root by the following formula. "Dentin moisture in percentage = (before desiccation weight - after desiccation weight)/ before desiccation weight × 100".

Digital grain moisture meter measurement reading was expressed as mean ± SD in each group and compared to the true value. The hypothesis was that the moisture content meter accurately records the true moisture content of dentin. Statistical significance (P-value < 0.05) was determined by one-way ANOVA with Dunnett's correction for multiple comparisons.

Results

Part 1

The coefficient of variation among repeated five readings was ranged between 0 and 0.0004 in six grain modes. Therefore, this machine, which was originally developed for six different grains, is reliable for dentin moisture measurements. Further, these non-destructive measurement steps described in Figure 1b, took less than 30 seconds.

Part 2

The time required to complete desiccation of dentin is summarized in Figure 2. The dentin moisture (weight) loss was rapid in the first hour, slow between one and five hours, and no change was recorded after five hours.

Parts 3 and 4

Co-relationship analysis between the six measuring modes in the digital grain moisture meter and the true moisture content are summarized in Table 1. The five readings for each sample were practically identical while the moisture content of each of the 17 samples varied resulting in the recorded standard deviations. The moisture meter recording at *polished rice* mode was significantly different from the true moisture content ($p>0.05$). This was also observed when measuring the effect of re-moisturizing the samples ($p>0.05$). It was noted that the *polished rice* mode of the meter cannot be used, whereas the other five modes appear reliable for dentin moisture measurement ($p<0.05$). It was also clear that it was not possible to regain the moisture loss in dentin by recondition the tissue at 100% humidity at 37 °C (Table 1).

Discussion

Orban mentioned that the moisture content in coronal dentin structure is 13.2 % 34. Papa et al. reported that vital dentin had a moisture content of 12.4 % whereas dentin from endodontically treated teeth had a moisture content of 12.1 % 25. Burnett and Zenewitz studied freshly extracted caries-free whole teeth without the pulp and dehydrated them successively in an oven, and its moisture contents was 10.0 % 35. Our results correspond to these reports.

We observed that dentin that was left to air dry even for a brief time period underwent irreversible moisture loss. It was not possible to re-hydrate these teeth completely at 100% humidity for 24 hours (Table 1). Apparently, once the moisture has been lost from dentin tissue, it can not completely recover its moisture content, even in a saturated atmosphere at body temperature. This finding suggests that excessive dehydration should be avoided when handling teeth to be used for dentin experimentation when moisture content is critical.

The question of how structural strength of the tooth in relate to dentin moisture is critical since the dentin serves as a substrate for most endodontic and restorative procedures. Huang et al. reported that substantial dehydration changes the fracture characteristics of dentin specimens 26. A range of biomechanical features such as the collagen crosslink content of dentin is affected by moisture 36. There is presently, however, no objective definitive proof of any relationship between mechanical weakening of dentin and moisture content. However, *in vitro* technique introduced in this study could help in studying these questions. Further, studying the impact of dentin moisture content on structural strength of teeth during bonding experimentation, *in vitro*, requires access to a simple and nondestructive method to objectively measure dentin moisture conditions 37. In these respects, there are applications for the findings of this study. There is a future research possibility to combine this technique with the dentin permeability system 38. Added pulpal pressure simulation may provide us

useful information which will lead to the next phase of the research with the goal of using impedance for rapid and non-destructive dentin moisture measurement in the clinical setting.

Conclusions

1. Each non-destructive measurement took less than 30 seconds.
2. 24 hours storage at 37 °C and 100 % humidity did not restore dentin moisture.
3. Five grain modes had a high validity, and thus can be used for dentin moisture measurements.

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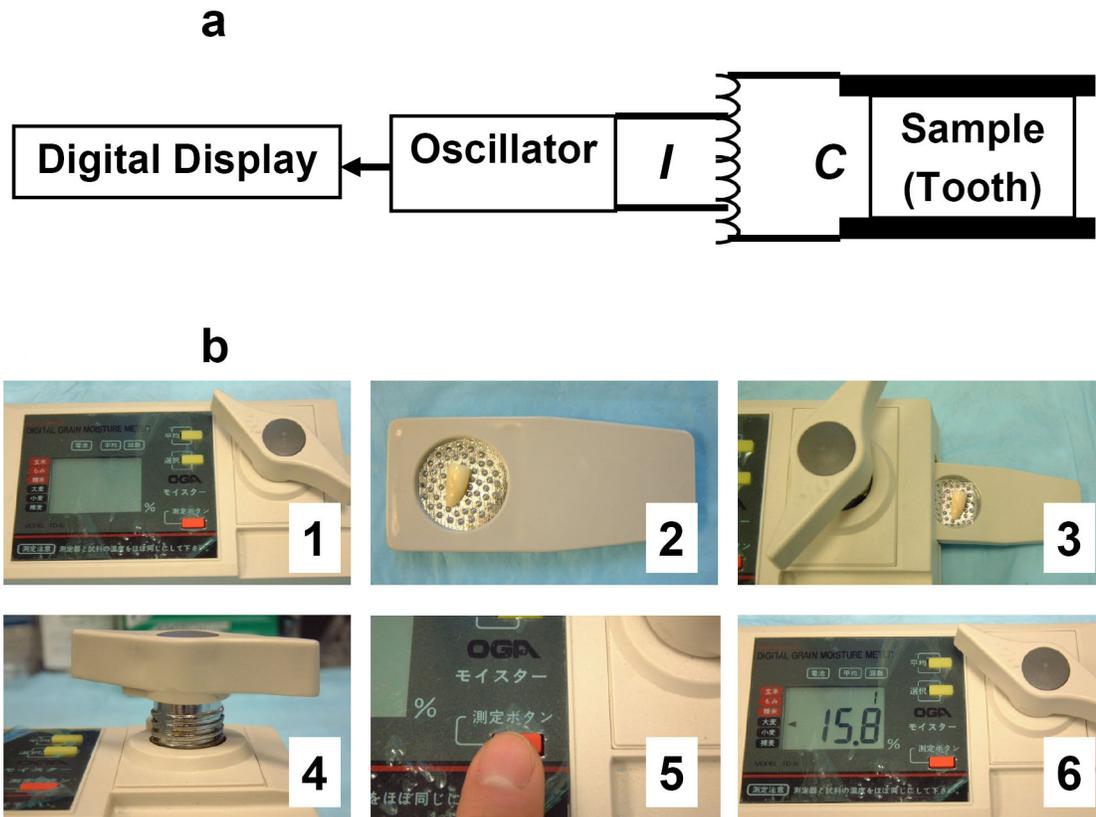
**Figure 1.**

Figure 1a

A map of the internal structure of the grain moisture testing machine (TA-5, OGA Electric, 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 to machine main body.
- 4; The screw chamber was tighten 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 digital displayed.

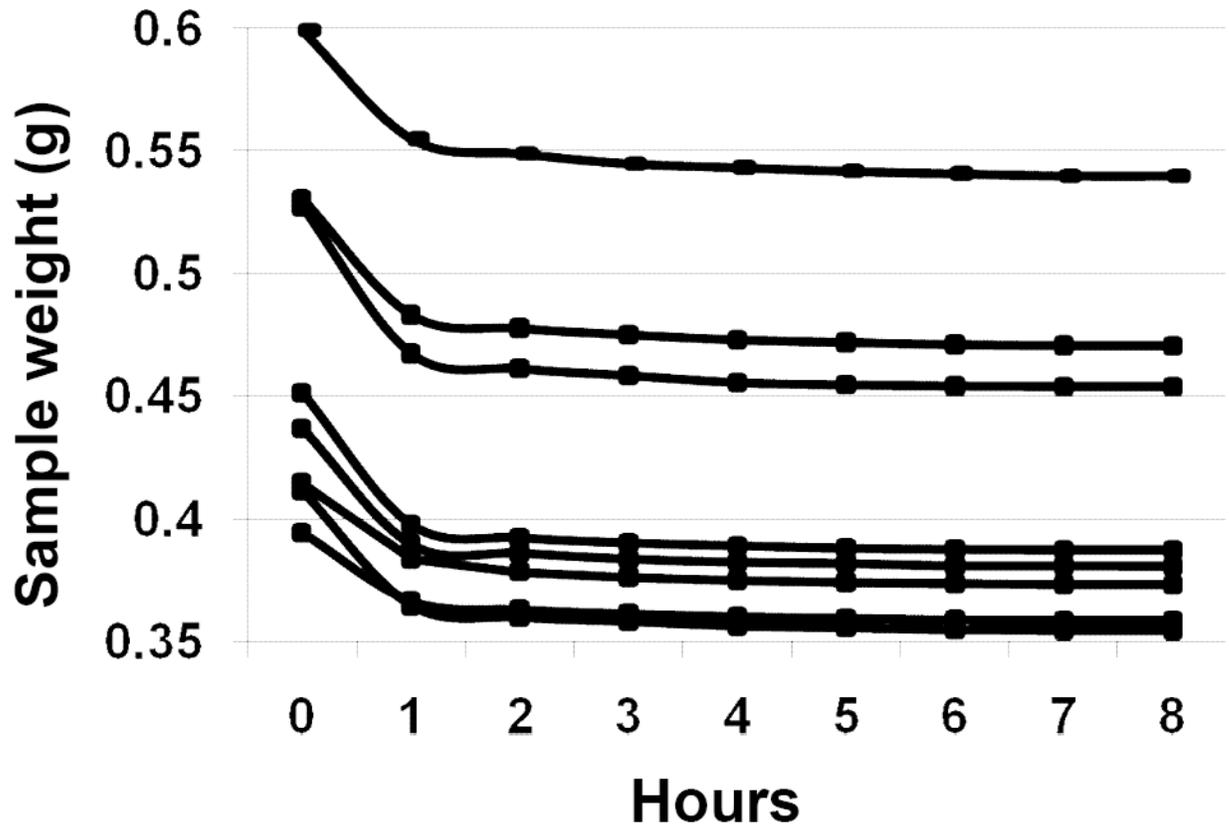


Figure 2.
Sample weight change in 8 hours in traditional desiccation methods of dentin moisture measurement

Table 1

Co-relationship analysis between the six measuring modes in digital grain moisture meter and true values

Time (hours)	Unpolished rice	Unhulled rice	Polished rice	Barley	Wheat	Rye	True moisture
Initial measurement	15.2 (2.1)	14.7 (1.9)	16.1 (2.4)*	13.3 (1.9)	14.3 (2.0)	13.9 (2.0)	13.2 (2.7)
Rehydrated	12.8 (2.3)	12.4 (2.3)	13.4 (2.4)**	11.1 (2.1)	11.9 (2.3)	11.8 (2.2)	10.8 (1.8)

Control was calculated by using traditional methods of weight change before and after desiccation

Average (S.D.); Average moisture % (Standard Deviation)

* Significant for Polished rice vs. control $p > 0.05$

** Significant for Polished rice vs. control $p > 0.05$