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The Influences of Dehydration on the Mechanical Properties of Human Dentin

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Abstract: The complex, dynamic, and hydrated microstructures of human dentin serve as the major determinant for the restorative performance of biomaterials. This study aimed to evaluate the mechanical properties of human dentin under different hydration conditions. The occlusal dentin of five third molars was exposed and cut into 1 mm^2 dentin slabs. The slabs were then polished and further cut into 1 mm^2 dentin beams and stored in distilled water. Two beams/tooth were used for testing their hardness (H) and elastic modulus (E) at 5 min (baseline), 1 h, and 24 h after dehydration (23 °C and 30% RH), and also for measuring weight at following dehydration times: 0 min, 5 min, 1 h, and 24 h. Five additional molars were employed to prepare 0.4 mm^2 dentin beams (3/tooth) for determining ultimate tensile strength (UTS) at 5 min (baseline), 1 h, and 24 h post-dehydration. Statistical significance was set at $\alpha = 0.05$. Dehydration time significantly affected H, E, weight-loss, and UTS of dentin ($p < 0.05$). H and E values showed a strongly positive and significant correlation ($r > 0.5$, $p < 0.05$). Dehydration can substantially modify the mechanical properties of dentin, leading to misinterpretation of restorative outcomes in vitro.

Keywords: dentin; dehydration; hardness; elastic modulus; correlation; ultimate tensile strength

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1. Introduction

Human teeth are composed of three mineralized tissues: cementum, dentin, and enamel. The cementum is an avascular connective tissue involved in the attachment of teeth to the bony cavity and covers the surface of the tooth root. Enamel is a highly mineralized structure that, in a healthy condition, covers the exposed part of the tooth, forming the anatomical crown. Dentin is a hydrated mineralized tissue that forms the bulk of a human tooth. The dentin and enamel's organization and composition construct a highly sophisticated mineral system designed to withstand specific mechanical stress types. Together with its unique hydration status, the complex structural organization of human dentin contributes to its biomechanical properties [1]. These properties, in

turn, act as major determining factors in almost all restorative procedures performed both in vitro and in vivo [2]. At the smallest length scales, dentin is composed of an organic matrix, primarily of type I collagen and an inorganic phase made up of carbonate-rich apatite crystal. However, these components are not uniformly distributed in dentin's microstructure, contributing to a hyper mineralized (~95 vol.%) tubular phase, called peritubular dentin (PTD), interposed to a less mineralized (~30 vol.%) collagen-rich fibrillar matrix phase termed intertubular dentin (ITD) [3]. More precisely, each mineralized fibril of ITD is a composite of a collagen framework together with plate-like apatite crystals with the c-axis co-aligned with the collagen fibril [4,5]. Although more mineralized than ITD, PTD contributes to only 10–20% of the human dentin [5], and therefore mechanical characteristics of human dentin mainly depend on the ITD matrix [6].

Laboratory bond strength tests are intended for preclinical screening of adhesives and restorative materials. Dehydration of bonded dentin specimens in vitro may increase bond strength and the number of cohesive dentin failures [7]. Since superior mechanical properties of the components forming the bonding interface of an adhesive restoration can be associated with higher durability of bonding and restoration [8–10], an apprehension of the mechanical characteristics of dentin such as hardness (H), elastic modulus (E), and ultimate tensile strength (UTS) is critical to predicting the outcome of the restorative procedures that involve dentin [11]. A nanoindenter is frequently employed for such assessments because the device allows the simultaneous measurement of H and E on small amounts of materials based on indentations' load-displacement data [12–15]. However, because of experimental complications associated with testing in liquid environments and keeping specimens wet during testing, most assays with nanoindenters to date have focused on dry dentin [16]. Important questions thus remain unanswered as to how hydration status influences dentin's mechanical properties.

Therefore, this study aimed to evaluate the H and E of human dentin having different hydration statuses. The null hypotheses tested were: (1) dehydration would not affect the H, E, and UTS of human dentin, and (2) there would not be any linear relationship between the H and E values of dentin.

2. Materials and Methods

This investigation was conducted following the Declaration of Helsinki of 1975, revised in 2013. The Ethical Committee of Hokkaido University Faculty of Dentistry approved the study protocol (Approval number 2018-9; Approval date 1 February 2018). An overview of the study design has been shown in Figure 1.

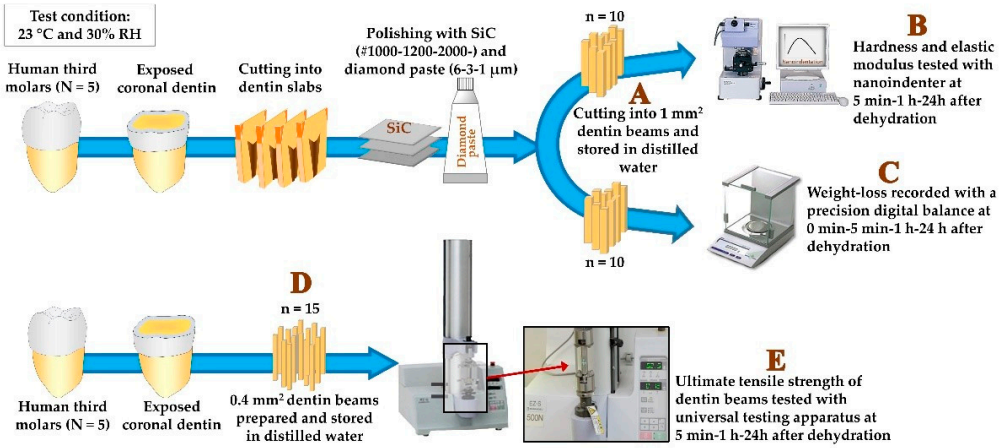


Figure 1. Schematic overview of the study.

2.1. Dentin Specimen Preparation for H and E Tests

Five human third molar teeth were used for this methodology. All teeth were collected after the patients' informed consent and stored in an aqueous solution of 0.5% Chloramine-

T at 4 °C. The teeth were free from any signs of caries, cracks, or fractures. Flat, occlusal dentin surfaces of the third molars were exposed, utilizing a gypsum model trimmer under a water coolant. Dentin slabs were then prepared by a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA). The slabs were then sequentially finished with 1000-, 1200-, and 2000-grit waterproof SiC paper (Sankyo-Rikagaku, Saitama, Japan) under running water; and polished with 6, 3, and 1 μm particle size diamond pastes (DP-Paste, Struers, Ballerup, Denmark) for 1 min each. This procedure contributed to a uniform thickness and smooth surface of the specimen essential for the H and E measurement precision. The specimens were cleaned in an ultrasonic unit (Fine ultrasonic cleaner, model FU-2H, Gao Hui Mechanical and Electrical International Trade Co., Ltd., Nanjing, China) with distilled water for 1 min after every finishing and polishing step. Subsequently, 1 mm^2 dentin beams were prepared from each polished specimen utilizing a low-speed diamond IsoMet saw (Figure 1A). Ten polished and cleaned beams (2 beams/tooth) were then placed in distilled water and used for H and E testing. The rest of the beams kept in distilled water were employed for weight-loss measurement in different dehydration time points.

2.2. H and E Tests by a Nanoindenter

Pilot studies were done to establish the testing parameters for the H and E tests of the dentin beams. It was confirmed that a maximum of 5 min could be necessary for removal, blot-drying, fixing, and placement of a stored specimen in the testing device before starting the test. Accordingly, in a sequential manner, ten polished dentin beams (2/tooth) were removed from distilled water, blotted of excess water (Kimwipe S-200, Nippon Paper Crecia Co., Tokyo, Japan), fixed on glass slides (Matsunami Glass Ind. Ltd., Osaka, Japan) and tested with a nanoindenter (DUH-211, Shimadzu, Japan) having a triangular pyramidal diamond indenter with a tip angle of 115° and a radius 0.1 μm (Figure 1B). The ITD at the center of each beam was targeted. Samples were tested at room conditions (23 °C and 30% RH). Indentations were performed at 5 min, 1 h, and 24 h after removal from water at a constant speed of 0.2926 mN/s, with a 45 s holding at peak load. Therefore, the 5 min values were considered as "hydrated"; and the rest of the values, which were obtained after 1 h and 24 h of dehydration (23 °C and 30% RH) were considered "dehydrated". The maximum depth of indentation was 1.2105 μm, which corresponded to the maximum loads of 5.04 mN. Data were discarded when a part of any indentation included a dentinal tubule. H and E values were obtained from the default software of the nanoindenter device. At least a 10 μm distance between adjacent indentations was maintained for all beams. Poisson's ratio assumed for dentin was 0.30.

2.3. Weight-Loss Measurement Tests for Dentin Beams

Ten polished dentin beams (2/tooth) were used to measure their weight-loss due to gradual dehydration in room conditions (23 °C and 30% RH). The dentin beams were removed from distilled water, quickly blotted off, and placed on the stage of a precision digital balance (AB204-S Analytical Balance, Mettler Toledo, Greifensee, Switzerland). The specimens' weight-loss was monitored over time and recorded at 0 min, 5 min, 1 h, and 24 h of free ambient dehydration with an accuracy of 0.1 mg (Figure 1C). Considering the water content of human dentin as being 10% by weight [17], the amount of water loss from the dentin beams (in mg) and water-loss percentage was also calculated.

2.4. Dentin Specimen Preparation for Ultimate Tensile Strength Test

Five additional human third molars free from any signs of caries, cracks, or fractures were used. All teeth were collected and stored in the same manner as mentioned for the H and E tests. Flat, occlusal dentin surfaces of the teeth were exposed using a gypsum model trimmer under water coolant. Dentin beams of 0.4 mm^2 size were prepared with IsoMet following the same procedure as mentioned before (Figure 1D). The size of the dentin beams was determined based on the results of a pilot experiment. The tendency of cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan) failure during

ultimate tensile strength (UTS) testing was least when the dentin beams covered a surface area of approximately 0.4 mm^2 or less. Prepared beams were collected and kept in distilled water before testing.

2.5. Ultimate Tensile Strength (UTS) Test

Three beams/tooth were tested at 5 min, 1 h, and 24 h after drying. After removal from water-storage, each beam was blotted with Kimwipe. For evaluating the UTS, the specimens were fixed with cyanoacrylate glue to Ciucchi's jigs and mounted to a universal testing apparatus (EZ-S, Shimadzu, Kyoto, Japan). The dentin beams were then subjected to tensile stress at a crosshead speed of 1 mm/min until failure (Figure 1E). After the fracture, the specimens were removed from the testing apparatus, and their cross-sectional areas were measured. The ultimate load to failure was recorded in Newton (N). The mean UTS values were then calculated by dividing the load (N) at which failure occurred by the cross-sectional area (mm^2) as follows: UTS (MPa) = Load (N)/Area (mm^2). It takes approximately 5 min to remove a beam from the storage medium, blot off the water, fix to the Ciucchi's jig with cyanoacrylate glue (Model Repair Blue II), and allow the glue to set before actual testing. Therefore, in the present study, the baseline of the UTS testing of the dentin beams was set at 5 min.

2.6. Statistical Analysis

The normality and homogeneity of H and E data were confirmed by using the Shapiro–Wilk and Levene's tests. The data were then analyzed by one-way ANOVA (Analysis of Variance) to evaluate the effects of dehydration on H and E values. Multiple comparisons were performed with Duncan's post hoc tests at a 5% level of significance. The bivariate Pearson Correlation test was also done to evaluate whether a statistically significant linear relationship exists between these two continuous variables (H and E). Weight-loss data of dentin beams were analyzed to confirm dehydration. The UTS data were analyzed using a Friedman test followed by Wilcoxon signed-rank test with Bonferroni correction (significance level, $p < 0.0125$). All statistical analyses were done by using SPSS 25.0 for Windows (SPSS, Chicago, IL, USA). For all analyses, the dentin beam was used as the statistical unit.

3. Results

3.1. H and E of Dentin Beams

The mean H and E values of the tested human dentin beams are shown in Table 1. One-way ANOVA demonstrated that dehydration exerted significant effects on dentin's H ($F = 40.149$; $p < 0.001$) and E ($F = 10.471$; $p < 0.001$).

Table 1. Mean hardness and elastic modulus ± standard deviation of human dentin beams in MPa (n = 10 beams).

Mechanical Properties	Dehydration Time		
	5 min (Baseline)	**1 h**	**24 h**
Hardness *	404.4 ± 84.2 A	488.1 ± 71.6 B	704.9 ± 75.9 C
Elastic Modulus *	17,311.0 ± 2742.3 A	20,256.0 ± 1292.2 B	21,324.0 ± 1784.8 B

* Comparisons are valid between different testing points for each tested property. Different upper case letters indicate statistically significant differences (Duncan's test, $p < 0.05$).

In general, the H and E values increased with increasing dehydration. Multiple comparisons with Duncan's test revealed that both H and E values increased significantly at 1 h and 24 h from the baseline (5 min; $p < 0.05$). Though all 24 h values were higher than 1 h values, a significant increase was particularly observed in the case of H ($p < 0.05$).

A Pearson Correlation test demonstrated a significant linear relationship between H and E ($p = 0.001$; Figure 2). The direction of the relationship was positive, meaning that

these variables tend to increase together (i.e., a greater H value is associated with a greater E value). The strength of the association was strong ($r > 0.5$).

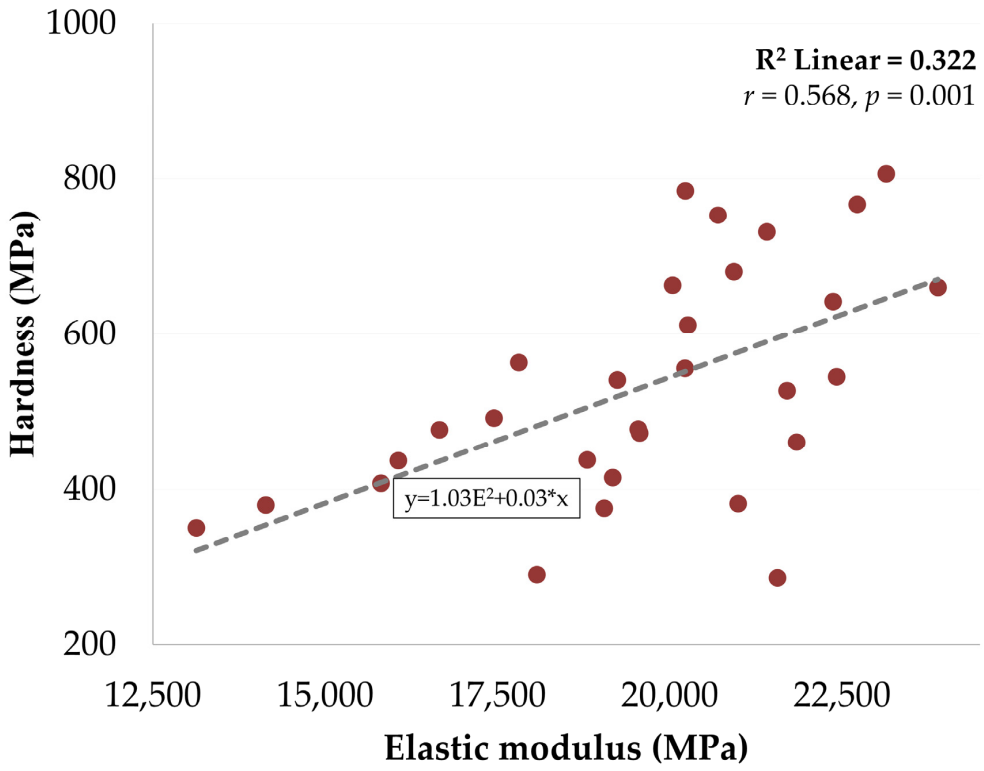


Figure 2. Pearson correlation test results showing significant ($p < 0.05$) and strong ($r > 0.5$) linear relationship between the Hardness and Elastic modulus values (in MPa) as demonstrated by hydrated (tested after 5 m of removal from storage) and dehydrated (tested after 1 h and 24 h of dehydration) human dentin beams.

3.2. Weight-Loss of Dentin Beams

The mean weight loss of the human dentin beams is shown in Table 2.

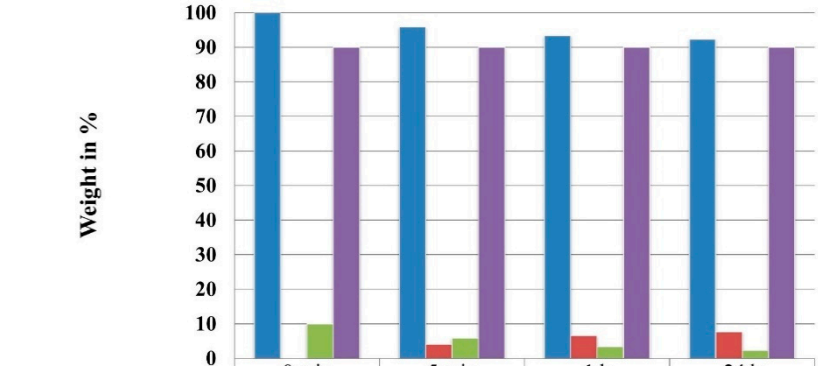
Table 2. Mean weight ± standard deviation of human dentin beams in mg (n = 10 beams) *.

Tested Material	Dehydration Time			
	0 min	**5 min**	**1 h**	**24 h**
Human Dentin Beams	10.25 ± 0.32 A	9.83 ± 0.35 B	9.57 ± 0.52 B	9.46 ± 0.50 B

* Comparisons are valid between different testing points. Different upper case letters indicate statistically significant differences (Duncan's test, $p < 0.05$).

One-way ANOVA demonstrated significant effects of dehydration on the weight-loss values ($F = 6.694$; $p = 0.001$). Duncan's post hoc test revealed significant weight loss of dentin beams due to dehydration at 5 min, 1 h, and 24 h ($p < 0.05$) compared with the 0 min values. Although gradual dehydration of the dentin beams was confirmed by their gradual weight-loss, the differences were not significant when 1 h and 24 h values were compared with 5 min values ($p > 0.05$).

The amount of water loss from the dentin beams and water-loss percentage are shown in Figure 3. The mean water loss percentage by weight of dentin beams at 5 min, 1 h, and 24 h were 4.1, 6.63, and 7.7, respectively.



	0 min	5 min	1 h	24 h
Total weight at	100	95.9	93.37	92.3
Water loss at	0	4.1	6.63	7.7
Remaining water at	10	5.9	3.37	2.3
Weight without water at	90	90	90	90

Figure 3. Water-loss percentage (%) of dentin beams calculated assuming that human dentin's water content is about 10% by weight [17].

3.3. UTS of Dentin Beams

There was a statistically significant difference between the UTS of dentin observed at different drying times, $\chi^2(2) = 10.133$, $p = 0.006$. A box-whisker plot of UTS is shown in Figure 4.

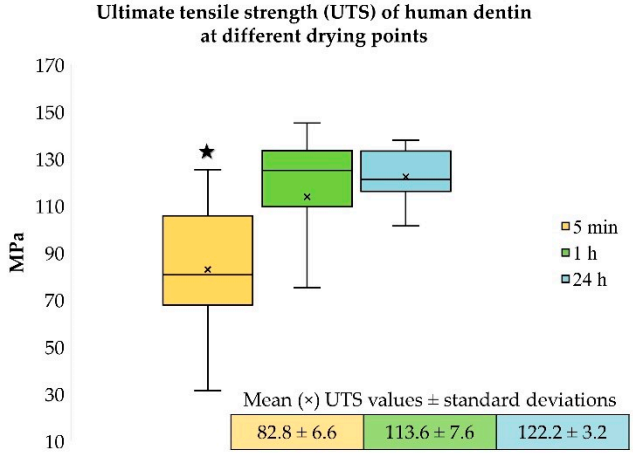


Figure 4. Box-whisker plot (minimum-(lower quartile-median-upper quartile)-maximum) of UTS values of human dentin at 5 min, 1 h, and 24 h after blot drying. The statistically significant difference between different time groups has been presented with the "★" symbol. The table in the figure shows the mean (×) UTS values of different groups with their standard deviations. An overall increase in the mean UTS can be observed with increased drying time.

Median (IQR) perceived UTS for the 5 min, 1 h, and 24 h drying were 80.5 MPa (67.7 to 105.6), 124.9 MPa (109.4 to 133.2) and 121.1 MPa (116.0 to 133.1), respectively. There were no significant differences between the 1 h and 24 h values ($Z = -0.568$, $p = 0.570$). However, there were statistically significant increases of UTS when 5 min values were compared with 1 h ($Z = -2.669$, $p = 0.008$) and 24 h ($Z = -3.237$, $p = 0.001$).

4. Discussion

Microtensile bond strength test has been most frequently utilized in the bond strength testing laboratories to assess the adhesive and restorative materials' bonding performance to teeth [18,19] after 24 h and long-term water storage. In this technique, bonded beams (or sticks) are subjected to a tensile load to induce bond failure. Therefore, the mechanical

properties of the components of the bonded assembly, such as dentin, can provide a preliminary understanding of the restorative outcome [2,8,20]. Accordingly, in the current study, we employed dentin beams to assess mechanical properties. As dentin's mechanical properties mainly depend on the ITD matrix [6], we used ITD to determine dentin's H and E.

A nanoindenter can simultaneously evaluate the H and E of a material by the Oliver and Pharr method [21]. Nevertheless, dentin's unique moist characteristic puts inherent challenges on testing because the nanoindenter device was designed for testing dry samples. Our pilot studies helped to address and overcome this drawback. To avoid dehydration, following the testing recommendations [22], dentin beams were withdrawn from the distilled water just before testing, and indentations were done 5 min after removal. Thus, the possibility of dehydration was kept to a minimum for the "hydrated" samples. However, the "dehydrated" samples were tested at 1 h and 24 h of dehydration (23 °C and 30% RH). Furthermore, to allow sufficient creep recovery of the hydrated samples, the holding time at peak load was set 45 s [16,23]. Our pilot study established that, similar to the μTBS test, the removal, blotting, fixing, and beginning of the indentation procedure can take around 5 min. Therefore, we started assessing the H and E after 5 min of removal from storage as a baseline time for testing.

In our study, gradual weight-loss of the tested dentin beams confirmed their dehydration in room conditions (Table 2; Figure 3). According to our nanoindentation test results (Table 1), hydration status significantly influenced dentin's H and E ($p < 0.05$). Moreover, dentin's UTS was also significantly affected by gradual dehydration ($p < 0.05$). Therefore, the first null hypothesis was rejected. Bertassoni et al. reported that with drying, dentin's collagenous matrix collapses, compressing the loose extrafibrillar mineral, this phenomenon increases the rigidity of the dentine surface, leading to higher surface H [24]. Similarly, in the present study, the mean H values increased significantly with drying at 1 h and 24 h ($p < 0.05$; Table 1). Marshall and coworkers [2] reported that H of ITD lies between 0.15–0.51 GPa. However, the H value increases to 0.6–0.7 GPa when samples are tested in completely dry conditions [3]. Mean H values obtained in the current investigation also lie within this range.

Previous studies reported that E values of ITD range from 17.7 to 21.1 GPa [2]. The E values obtained in our study in hydrated and dehydrated conditions are also within this range and show a significant gradual increase at 1 h and 24 h ($p < 0.05$; Table 1). Moreover, Oyen ML reported that in the case of mineralized tissues, with the increase of H, E also increases [25]. In an indentation technique, H is a measure of the material's resistance to deformation by surface indentation. On the other hand, E is the ratio of stress to strain when deformation is totally elastic. However, in the case of mineralized tissues, the contributions from elastic and plastic deformation can be similar. Therefore, H is directly dependent on E. This means, an increase in E contributes to a rise of H. Angker et al. found similar results when they examined carious primary dentin [26]. Chang et al. and Zysset et al. reported similar findings with alveolar bone and femur [27,28]. Based on their observations, they concluded that plastic deformation resistance in mineralized tissue could be a direct function of the tissue elastic modulus. Our results are in agreement with these observations. A strong, significant, and positive linear relationship between H and E ($p = 0.001$; Figure 2) meant that a greater H value was associated with a greater E value, rejecting the second null hypothesis.

Although a predominance of cohesive dentin failure during bond strength test at laboratory settings indicates higher bond strengths [29], it may also result from dehydration of dentin producing higher ultimate tensile strengths and leading to overestimating the bonding performance. However, in clinical situations, dentin dehydration does not occur in the oral cavity. Therefore, to avoid dehydration, tooth substrates should be immersed in aqueous media or be covered with wet tissue papers from the point of extraction till the end of bond strength testing.

The nanoindentation tests provide mechanical data for a surface stress response (H and E), while the weight measurements used in the calculation of water loss represent a bulk character (1 mm^2). In this sense, the leakage gradient of dentin from the surface to the interior of the whole sample may play a critical role in the dehydration process at higher times (24 h), the probable reason why the 24 h H value was so high. On the other hand, the major difference observed between the nanoindentation test results with respect to the UTS data may be due to this difference in dehydration gradient considering the bulk dentin, although the small dimensions of the beams used for UTS testing (0.4 mm^2) tend to minimize these differences. However, the information can be considered valuable in a different way if we consider the adhesive response of the surface (nanoindentation test) and the mechanical response of an adhesive restoration (cohesive failures) at different times in in vitro studies.

5. Conclusions

Within the limitations of the present study, we conclude that dehydration of human dentin results in the increase of elastic modulus and concurrent increase of hardness, and enhances the ultimate tensile strength. Dehydration can substantially modify the mechanical properties of dentin, constituting a critical factor in the interpretation of in vitro restorative studies.

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