©Operative Dentistry, 2006, **31-5**, 536-542

Laboratory Research

Influence of Bleaching Agents and Desensitizing Varnishes on the Water Content of Dentin

H Betke• E Kahler • A Reitz
G Hartmann • Á Lennon • T Attin

Clinical Relevance

The dehydration of tooth structure is discussed as a possible factor for tooth hypersensitivity arising during bleaching; however, it still remains unclear to what extent glycerine-based bleaching gels dehydrate dentin and whether dentin hydration can be maintained by protective dentin varnishes.

SUMMARY

This *in vitro* study investigated the possible dehydration of dentin caused by bleaching agents. Furthermore, it tested whether protective dentin varnishes can maintain the physiological moisture of dentin during bleaching treatment. Fifty-five standardized dentin cylinders were prepared from freshly extracted bovine incisors under constant water irrigation. Prior to bleaching, the treatment specimens were conditioned at room temperature in a hygrophor for 14 days. The samples were divided into 11 groups. The Group A specimens, which were completely dehydrated, and Group B, which was stored for 2 weeks in a hygrophor, served as controls (A, B n=5). The other samples (n=10 each group) were coated with Vivasens [VS] (C), Bilfuorid [BF] (D) and Seal&Protect [SP] (E). Five specimens from each group (C-E) were subsequently treated with an experimental bleaching gel (Exp BG) (20% carbamide peroxide [CP], glycerine-based gel): Cb, Db, Eb. The remaining specimens were bleached with Exp BG (F) only, Vivastyle (G: 16% CP, glycerine-based gel) or Vivastyle Paint On (H: 6% CP-varnish) for 7 days (n=5 each group) with bleaching time for gels: 2 hours/day, paint on: 20 minutes/day. After the respective treatments, the overall water content of each specimen was determined using the analytical method of Karl-Fischer-titration. The water content of bovine dentin (Group B, mean%±SD) obtained in this

*Herbert Betke, Dr med dent, assistant professor, Department of Operative Dentistry, Preventive Dentistry and Periodontology, Georg-August-University Goettingen, Goettingen, Germany

Elke Kahler, Dr, research assistant, Department of Medical statistics, Georg-August-University Goettingen, Goettingen, Germany

Angelika Reitz, laboratory assistant, Department of Geochemistry, Georg-August-University Goettingen, Goettingen, Germany

Gerald Hartmann, Dr, assistant professor, Department of Geochemistry, Georg-August-University Goettingen, Goettingen, Germany

Áine Lennon, Dr med dent, research assistant, Department of Operative Dentistry, Preventive Dentistry and Periodontology, Georg-August-University Goettingen, Goettingen, Germany

Thomas Attin, Dr med dent, professor and head of Department of Operative Dentistry, Preventive Dentistry and Periodontology, Georg-August-University Goettingen, Goettingen, Germany

*Reprint request: Robert-Koch-Str 40, 37075 Goettingen, Germany; e-mail: hbetke@med.uni-goettingen.de

DOI: 10.2341/05-89

study amounted to 15.24±0.4. All bleaching products significantly reduced the water content compared to the controls (exp BG: 13.32±0.47, Vivastyle 13.2±0.27, paint on 13.72±0.54; $p \leq 0.05$). Also, application of SP before bleaching resulted in reduced water content (14.06±0.12; $p=0.0005$). However, bleaching with exp BG following use of VS (14.99±0.42) or SP (13.85±0.26) did not result in a reduction of water content in dentin. Pretreatment with BF did not protect dentin from water loss during bleaching (12.44±0.38; bi $p=0.0009$). All glycerine-based bleaching products used in this study had a significant dehydrating effect on dentin. The application of protective varnishes prior to bleaching treatment may reduce or even prevent dentin dehydration.

INTRODUCTION

Bleaching is an effective method to lighten intrinsically stained or discolored teeth. Tooth hypersensitivity and/or gingival irritation might appear as negative side effects during the external bleaching of vital teeth.[1-5] In a report on nightguard vital bleaching, side effects were reported to affect up to 67% of all patients treated with this bleaching method.[1] In some experimental findings, small amounts of hydrogen peroxide were detected in the pulp after application of tooth bleaching agents on vital teeth.[6] The penetration of hydrogen peroxide into to the pulp chamber was discussed[7] as a reason for tooth hypersensitivity and mild inflammatory reactions of the pulp tissue. Usually, such hypersensitivities ceased soon after completion of the bleaching procedure.[4,8-10] Side effects from the bleaching procedure, such as tooth hypersensitivity, may be related to low water content of the bleaching agent, resulting in dehydration of the dental hard tissue.[10] It could be speculated that anhydrous-based whitening solutions may cause more side effects, because of their drying effect on teeth.

It is conceivable that protective or desensitizing varnishes applied prior to bleaching may prevent water loss from dentin by dehydrating bleaching agents. Varnishes, such as Vivasens, Seal&Protect or Bifluorid, did not compromise the bleaching effect of subsequently applied bleaching gels.[11] These varnishes led to a coverage and closure of open dentinal tubules, resulting in mechanical obliteration of the dentinal tubules or precipitation of calcium fluoride or proteins. Human dentin contains about 11% water, as determined by gravimetrical methods. In these experiments, water was eliminated from the teeth under vacuum[12-15] or by heating up to 150°C.[16-17] Individual values ranged between 8%-16%, depending on the specific conditions of each investigation (method of sample preparation, temperature and atmospheric pressure). Analytic water determination, according to the Karl-Fischer method [KFT],[18] is a widely used titrimetric method for water determination of various substances. It is also applicable to solid substances, such as dental hard tissue. Briefly, the moisture of dentin is chemically converted to a reaction product, which is recorded, giving the water content of the substrate. This study used the Karl-Fischer-Method for evaluating whether bleaching agents may lead to a suspected water loss and dehydration in (bovine) dentin. Furthermore, the authors planned to examine whether protective varnishes have the potential to prevent water loss from dentin during bleaching treatment.

METHODS AND MATERIALS

The design of this study is presented in Figure 1. Thirty bovine permanent incisors were extracted immediately

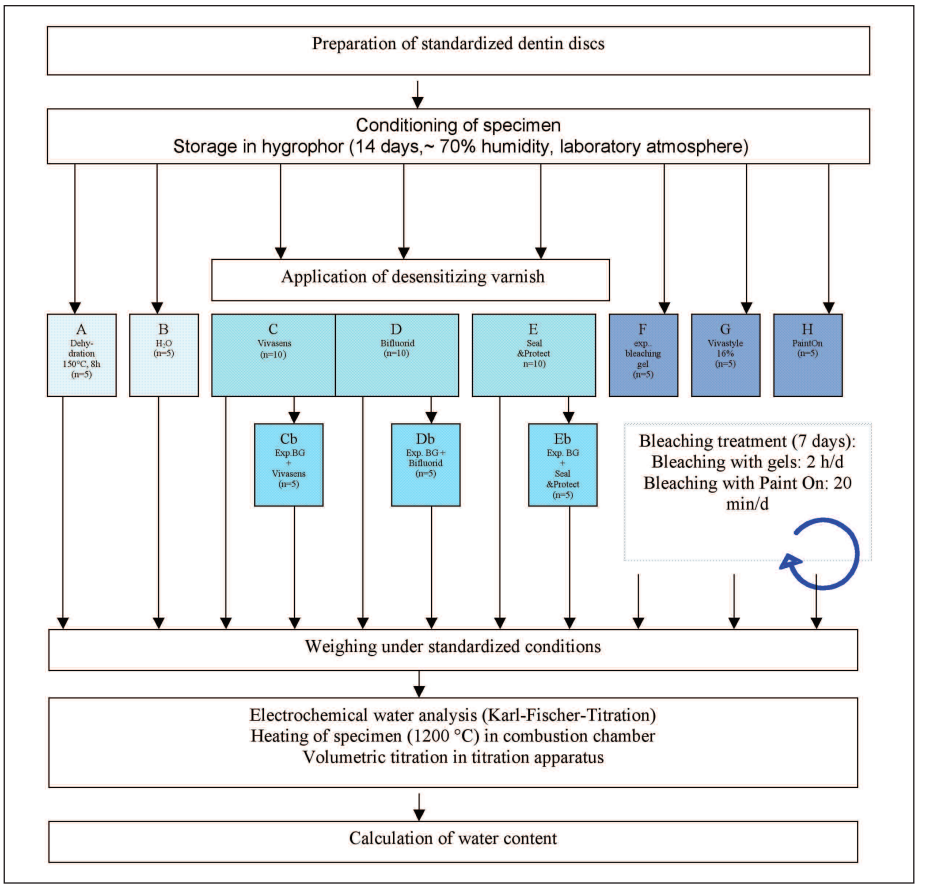


Figure 1. *Design of the study.*

after the animals had been killed, and the teeth were stored in saline solution (0.9%). Subsequently, 55 specimens were prepared from the cervical third of the roots, using a specific electroplated diamond core drill bit (5-mm diameter; custom-made product of Brassler/Komet, Lemgo, Germany) in a drilling apparatus (Metabo GmbH, Nürtingen, Germany) at 300 rpm under constant irrigation with saline solution (0.9%). In this first preparation step, a cylindrical specimen, containing the root canal in its center, was obtained in a vestibular-oral direction from each tooth (Figure 2). Separation of the cylinder into 2 parts was then performed using a separating diamond bur (ISO 806 314 165 524 010; Brassler/Komet, Lemgo, Germany) in a contra-angle handpiece under constant irrigation with purified water (Ampuwa, Fresenius Kabi, Bad Homburg, Germany). Subsequently, the surfaces of each specimen were flattened using a grinding and polishing apparatus (DP-U3, Struers, Erkrath, Germany) at 300 rpm under constant tap water irrigation. As a result of these preparation steps, 5-mm diameter and 1-mm thick disc specimens were obtained from the central root dentin, and the disks were checked with a micrometer (Digimatic; Mitutoyo-Meßgeräte, Leonberg, Germany). The samples had a weight of about 0.004 g, as recorded with a microanalytic scale (Sartorius, Göttingen, Germany). Prior to the experiments, the specimens were conditioned at room temperature (24.7-27.6°C) in a hygrophor for 14 days (at ~70% humidity). Furthermore, the specimens were stored in the hygrophor for an additional 7 days and were only removed from the hygrophor for their respective treatments.

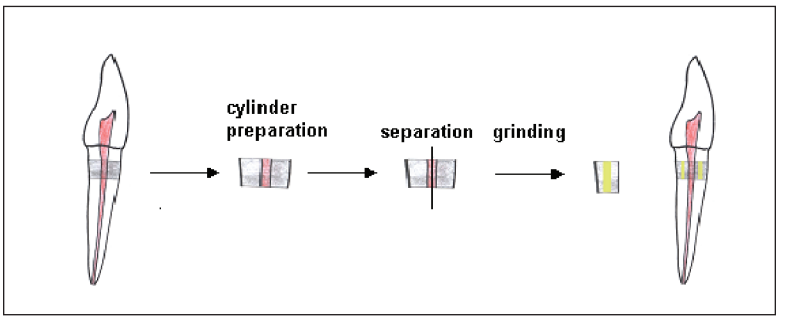


Figure 2: *Preparation of specimen (yellow marking: dentin disc specimen used for analytic investigation).*

Group A and B specimens were not further treated (varnished or bleached) and served as controls (A/B, n=5). The Group A specimens (n=5) were dehydrated for 8 hours at 150°C in a sterilizing oven (Kugelsterilisator, Hager&Werken, Duisburg, Germany) prior to water determination. The control specimens in Group B were not treated and were stored in the hygrophor.

Group C, D and E specimens (n=10) were treated with desensitizing or protective varnishes. The varnishes Vivasens (C), Bifluorid (D) and Seal&Protect (E) were applied evenly on specimen surfaces according to manufacturers' instructions. Within each group (C, D, E) 5 specimens were subsequently bleached with an experimental bleaching gel [Exp BG] (Cb, Db, Eb, n=5) before final water determination. The formulation of the gel is given in Table 1. Further specimens (Groups F, G, H, n=5) were only bleached with an experimental bleaching gel (F), Vivastyle 16% (G) or with Vivastyle Paint On (H) and were not treated with a desensitizing agent prior to bleaching (Table 2). The water composition of these samples was also determined by Karl-Fischer-Titration.

Bleaching was performed for 7 days. Exp BG and Vivastyle were applied on the complete surface of specimens in a 1-mm thick layer, with a 2-hour bleaching time. The paint-on varnish was applied in a thin layer for 20 minutes. Subsequently, the bleaching products were meticulously removed from the surfaces with a foam pellet (Pele Tim, Voco, Cuxhaven, Germany) and purified water (Ampuwa, Fresenius Kabi, Bad Homburg, Germany). Excessive water was carefully removed with a cellulose cloth (Hake-Kimberley, Mainz, Germany). Apart from application and removal of the bleaching agent or protective varnish, the specimens were stored in the hygrophor for 14 days. Finally, water analysis of the dentin discs was conducted.

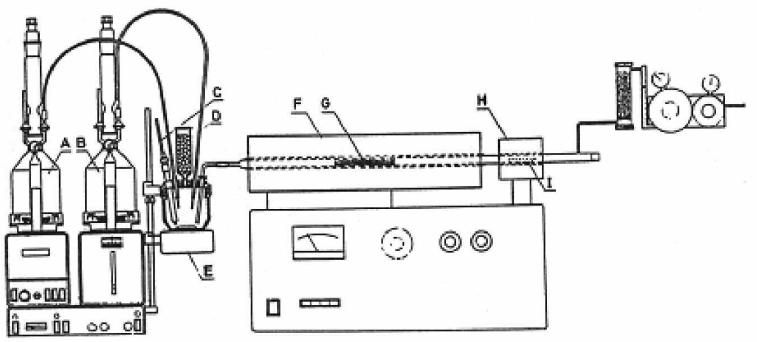
The overall water content of the specimens was determined by heating up to 1200°C (custom made KF oven;

Table 1: *Formulation of Experimental Bleaching Gel (20% carbamide peroxide)*

Component	% by weight
Glycerine (86%)	72.0
PEG600	3.0
Saccharine sodium	0.5
Sodium citrate	3.0
Silica	0.5
Flavor	0.5
Carbamide peroxide	20.0
Carbomer	0.7

Table 2: *List of Materials*

Vivasens [VS]	Ivovlar-Vivadent, Schaan, Liechtenstein
Bifluorid [BF]	Voco, Cuxhaven, Germany
Seal&Protect [SP]	Dentsply DeTrey, Konstanz, Germany
Vivastyle 16%	Ivovlar-Vivadent, Schaan, Liechtenstein
Vivastyle Paint-On	Ivovlar-Vivadent, Schaan, Liechtenstein
Experimental bleaching gel [Exp BG]	Incos, Nieder-Olm, Germany



A: vessel filled with Karl-Fischer solution
B: vessel filled with solvent (water-free methanol)
C: titration vessel with platin electrode
D: drying tower
E: magnetic stirrer
F: burner
G: burning chamber with calatyst
H: burner
I: dentin disc sample

Figure 3: *Schematic illustration of the measuring apparatus for Karl-Fischer-titration.*

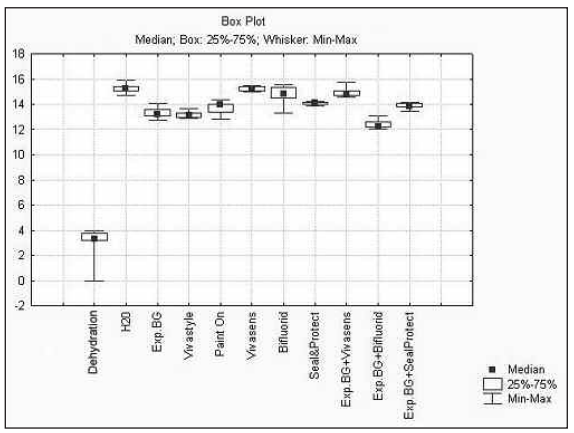


Figure 4: *Box plot illustration of results.*

Heraeus GmbH, Hanau, Germany). The expelled water was conveyed to the automatized KF-Titrator (KFD-Titrino 758, Titration stand 703; Metrohm, Herisau, Switzerland). Immediately before heating, the weight of each specimen was determined with a standard analytical balance to the nearest 0.1 mg (Sartorius, Goettingen, Germany) under standardized ambient conditions (25°C). Calculation of the overall water content was computed automatically by the titration apparatus software of the titration apparatus.

Determination of the water content, based on the following stoichiometrical reaction, is described by Bunsen[19]: $SO_2 + 2\ H_2O + J_2 \rightarrow H_2SO_4 + 2\ HJ$. Karl Fischer[18] discovered that this reaction could be used for water determination in a non-aqueous system containing an excess of sulfur dioxide. Water-free methanol proved to be suitable as a solvent. In order to achieve an equilibrium shift to the right, it is necessary to neutralize the acids that are formed during the process (HI and H_2SO_4) by organic bases (for example, pyridin). In this experiment, the specimens were heated up to 1200°C in a quartz tube burning chamber and an N_2 atmosphere (Figure 3). The quartz tube filled with a silver permanganate katalysator was preheated to 560°C before combustion took place (Figure 3). The overall water content released was conveyed to the titrator and titrated by use of the KF-solution. As a solvent, water-free methanol containing sulfur dioxide and pyridin as parts of reactants for titration was used. The titration vessel contained a platinum electrode for detecting a sharp increase in current flow or an abrupt potential difference (electrometric dead stop method). At this titration dead stop, an excess of iodide is produced. The titrator was calibrated with a reference sample (Pikrit, basic: model 600, probe 03.3000, consisting of standard clastic rock 5-30 cm with a known water content: 9.81%). A computer connected to the titration apparatus recorded the water content of the dentin specimens as percentage. Statistical analysis of the data was carried out using multiple pairwise-tests. Significance was set after Bonferroni correction at $p \leq 0.05$.

RESULTS

The water content (%) of the specimens for each experimental group is given in Figure 4. The overall water content of the control bovine dentin specimens (B) amounted to 15.2%±0.44. In the dehydrated specimen (A), only minimal amounts of residual water were detected (3.3%±1.61). The Group B control specimens had higher water content than the Group A control specimens (p=0.0000). The water content of the Group A controls was significantly lower compared to all other groups.

The application of bleaching products (F: experimental bleaching gel, G: Vivastyle 16%, H: Vivadent Paint On) on non-varnished dentin caused a significantly reduced water content of bovine dentin compared to the B control (F/B p=0.00024, G/B p=0.00003, H/B p=0.0019). However, the use of different bleaching agents (F, G, H) did not result in a different dehydration of the samples.

The application of protective varnishes Vivasens (C) and Bifluorid (D) did not lead to significantly lower water content as compared to the B controls (C/B p=0.93, D/B p=0.2624). Seal&Protect caused significant dehydration as compared to the B control (p=0.000469).

For combined treatment (the use of a bleaching agent after applying the protective varnish), some changes in water content were recorded compared to specimens that were bleached only, depending on the specific product; that is, specimens coated with Bifluorid showed a significant loss of water after bleaching with Exp BG (Db) compared to the coated, unbleached Group D specimens (D/Db p=0.000916). This dehydration was not observed for specimens first varnished with Vivasens (Cb) or Seal&Protect (Eb) (C/Cb p=0.3368, E/Eb p=0.1845).

DISCUSSION

For accurate water determination of specimens, many factors had to be taken into consideration. Environmental conditions, such as temperature, humidity and atmospheric pressure, influence the results. Also, technical difficulties of tissue extraction or tissue separation play an important role. Furthermore, differences among specimens, due to different composition, may have an impact on the outcome of results. The authors used dentin obtained from bovine permanent incisors. The animal pedigrees were very similar due to origin of cattle from the same farm. Therefore, it can be hypothesized that the structure and composition of the animals' teeth were very similar. Bovine dentin is frequently used as a substitute for human dentin for *in vitro* studies in adhesion, erosion and biomechanics,[20-22] because it is readily available and has a similar composition and chemical structure. Knowing that many variables influence the water content of dentin, the authors gently extracted the bovine dentin disc specimens from the roots and standardized the preparations as much as possible. Nevertheless, water loss during specimen extraction from the roots cannot be completely excluded. It was reported that dentin might be be rehydrated and the potential for rehydration increases at lower temperatures. However, according to Burnett,[12] it is elusive to restore the original water content of teeth and tooth structures to their former state of hydration. Therefore, storage in a hygrophor was performed prior to the experiment for moisture conditioning and compensation of water loss that occurred during specimen preparation.

The water loss at room temperature is fast and nonlinear, and a substantial proportion of the water in dentin may be lost within minutes due to dehydration.[23] Therefore, transfer of the samples from the hygrophor to the analytical scale was conducted in a standardized manner for all samples. Therefore, absolute values obtained in this study should be interpreted carefully but should allow for comparison among the different experimental groups.

The total water content of dentin can be divided into free water and bound water (structurally incorporated water). In the literature, the free water content of dentin was mostly investigated by determining the weight loss gravimetrically.[12,14,24-25] There are different approaches to investigating free and bound water fractions by Differential Thermal Analysis [DTA][26] or quantitative infrared spectroscopical analysis.[27] A disadvantage to the DTA and spectroscopic methods is the qualitative changes that occur in the content of bound water. In contrast, the thermobalance method[28] is able to register qualitatively and quantitatively. Additionally, the analytic KFT method, as used in this investigation, is able to detect water exclusively independent of other volative components, such as CO_2 or unspecified organic gases. According to Fischer,[18] the analytical method described can be basically applied to investigate the water contents of solids. The overall water content of bovine dentin obtained with the applied Karl-Fischer-methodology was 15.2%. This value is slightly higher than the average outcome by Papa and others,[25] who investigated the water content of human dentin thermogravimetrically. However, in this study, inter-individual variabilities were high, also giving higher values compared to the results of this study. Additionally, all bound water was not regarded by Papa and others,[25] which might explain the marginally lower values compared to the values from this study. As mentioned above, human dentin contains approximately 11% water, but the individual values range between 8% and 16%, depending on methodical differences.[29] Reviewing the literature, no study dealt with the difference in moisture content between human and bovine dentin. To test the applicability of the KFT method, the control specimens (A) were extremely dehydrated. The results of this study (3.3%±1.6) were consistent with the values found in the literature (5.5%±0.55).[29] The minimally lower values in this study might be explained by the more extreme dehydration treatment chosen. Nevertheless, the results of the control group showed that the KFT method is highly applicable to determine the moisture content of dentin.

It was suggested that bleaching agents used for external bleaching may dehydrate dental hard tissue. The subsequent rehydration, due to saliva contact, needs 1-2 weeks after bleaching and results in a slight color change of bleached teeth.[29] It is also conceivable that, dehydration due to bleaching agents, might be responsible for the reduced fracture toughness of previously bleached enamel.[30-31] Also, the dehydration effect of bleaching agents was discussed as a possible reason for the development of tooth hypersensitivity during bleaching therapy.[4] Additionally, side effects may also be related to water content of the respective bleaching solutions.[10] In many cases, glycerine is used in bleaching gels as a vehicle for active peroxide substances. Glycerine, on its own, can absorb water and is dehydrating in high concentrations, and, thereby, irritating to skin and mucous membranes.[32] In this study, an

experimentally formulated bleaching gel was tested as a control against commercially available products. This gel contained high amounts of glycerine (86%) and a few other ingredients usually also contained in bleaching gels (Table 1). Interestingly, all tested bleaching products led to dehydration, showing no difference in capability to dehydrate the dentin discs. According to the manufacturer, the bleaching product Vivastyle Paint On is not glycerine-based but contains ethanol as a solvent. It can, therefore, be assumed that ethanol, also similar to glycerine, might promote dehydration of dentin.

With respect to protective varnishes, it was found that Seal&Protect significantly desiccates dentin. No changes in dentin moisture were observed after application of Vivasens or Bifluorid. Seal&Protect is formulated very similarly to acetone-based dentin adhesives, such as Prime&Bond NT (Dentsply De Trey, Konstanz, Germany). Acetone is known to act as a water-chasing agent, removing water from dental hard tissue.[33] Therefore, it seems obvious that the acetone in Seal&Protect caused dehydration of the dentin discs in this study.

The combined treatment with protective varnishes, followed by the bleaching agents of specimens, showed remarkable results. In contrast to Bifluorid, coating specimens with Vivasens or Seal&Protect before bleaching therapy seemed to reduce the dehydrative potential of the bleaching agents. Nevertheless, the initial dehydration of Seal&Protect prior to bleaching also has to be considered. During the experimental procedure, it was observed that the Bifluorid coat peeled off from the specimen surfaces after a few days. It is possible that the remnants of the Bifluorid layer and the calcium-fluoride precipitate cannot inhibit the desiccation by bleaching agents. The Vivasens and Seal&Protect varnishes are resin-based agents that form an adhesive layer on specimen surfaces. According to manufacturers' information, these varnishes also form "tags" that can obstruct dentinal tubuli. Possibly, this pattern might stop a fluid movement through the dentin tubules and prevent further dehydration by the bleaching agents. It is interesting that the moisture of dentin was not reduced by bleaching after Vivasens application, although Vivasens contains an organic solvent (ethanol).

As discussed, dehydration might be a factor influencing the development of hypersensitivities during the bleaching of teeth. The exact mechanisms were not really understood until now. Matthews and others[34] assumed that evaporative water loss in dentin may be induced by air blasting, thereby removing fluid from the pulp-dentin complex, which might cause a disruption in odontoblasts and changes in pulpal blood flow. It still remains spectulative whether dehydration due to bleaching agents has similar effects.

The results of this *in vitro* study were obtained from non-vital teeth, which were kept in a humid environment during the experiment. The *in vivo* moisture of dentin could also be replaced by dentin fluid from the pulp. It is, therefore, conceivable that the net water content of dentin under *in vivo* conditions changes to the same extent observed in this study.

CONCLUSIONS

1. The results of this *in vitro* study show that differences in the water content of dentin can be investigated by the Karl-Fischer-Titration technique as a specific method for water determination of solid samples.
2. All glycerine-based bleaching products used in this study had a significant dehydrating influence on bovine dentin.
3. It can be assumed that a sole application of Seal&Protect may reduce the moisture content of bovine dentin. This observation was not true for the 2 protective varnishes Vivasens and Bifluorid.
4. The application of Vivasens or Seal&Protect prior to bleaching may reduce or even prevent the dehydration of dentin due to bleaching.

Acknowledgements

The authors are grateful to Mr Joerg Stange for making the bovine teeth available. This study was supported by material and a grant from Ivoclar Vivadent, Schaan, Liechtenstein.

(Received 14 June 2005)

References

1. Haywood VB, Leonard RH, Nelson CF & Brunson WD (1994) Effectiveness: Side effects and long-term status of Nightguard Vital Bleaching *Journal of the American Dental Association* **125(9)** 1219-1226.
2. Jorgensen MG & Carroll WB (2002) Incidence of tooth sensitivity after home whitening treatment *Journal of the American Dental Association* **133(8)** 1076-1082.
3. Haywood VB, Caughman WF, Frazier KB & Myers ML (2001) Tray delivery of potassium nitrate-fluoride to reduce bleaching sensitivity *Quintessence International* **32(2)** 105-109.
4. Leonard RH, Haywood VB & Philips C (1997) Risk factors for developing tooth sensitivity and gingival irritation associated with Nightguard vital bleaching *Quintessence International* **28(8)** 527-534.
5. Leonard RH Jr (1998) Efficacy, longevity, side effects, and patient perceptions of Nightguard vital bleaching *Compendium of Continuing Education in Dentistry* **19(8)** 766-770,772,774.
6. Thitinanthapan W, Satamanont P & Vongsavan N (1999) *In vitro* penetration of the pulp chamber by three brands of carbamide peroxide *Journal of Esthetic Dentistry* **11(5)** 259-264.

7. Cooper JS, Bokmeyer TJ & Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *Journal of Endodontics* **18(7)** 315-317.
8. Barnes DM, Kihn PW, Romberg E, George D, DePaola L & Medina E (1998) Clinical evaluation of a new 10% carbamide peroxide tooth-whitening agent *Compendium of Continuing Education in Dentistry* **19(10)** 968-972, 997-998.
9. Nathanson D (1997) Vital tooth bleaching: Sensitivity and pulpal considerations *Journal of the American Dental Association* **128 Supplement** 41S-44S.
10. Yarborough DK (2004) The safety and efficacy of tooth bleaching: A review of the literature 1988-1990 *Compendium of Continuing Education in Dentistry* **12(3)** 191-196.
11. Betke H, Revas P & Attin R (2005) [Einfluss von Desensibilisierungslacken auf die Zahnaufhellung in der Bleichtherapie] *Quintessence* **6** 589-597.
12. Burnett GW & Zenewitz J (1958) Studies of the composition of teeth VII. The moisture content of calcified tooth tissues *Journal of Dental Research* **37(4)** 581-589.
13. Brauer GM, Termini DJ & Burns CL (1970) Characterization of components of dental materials and components of tooth structure by differential thermal analysis *Journal of Dental Research* **49(1)** 100-110.
14. Lim JJ & Liboff AR (1972) Thermogravimetric analysis of dentin *Journal of Dental Research* **51(2)** 509-514.
15. LeFevre M & Manly RS (1938) Moisture, inorganic and organic contents of enamel and dentin from carious teeth *Journal of the American Association* **25** 233-242.
16. Holager J (1970) Thermogravimetric examination of enamel and dentin *Journal of Dental Research* **49(3)** 546-548.
17. Komrska J (1972) Derivatographic analysis of dentin *Journal of Dental Research* **51(1)** 148-150.
18. Fischer K (1935) [Neues Verfahren zur maßanalytischen Bestimmung des Wassergehaltes von Flüssigkeiten und festen Körpern] *Angewandte Chemie* **48** 394-396.
19. Bunsen R (1853) [Ueber eine volumetrische Methode von sehr allgemeiner Anwendbarkeit] *Annalen der Chemie und Pharmacie* **86** 265-291.
20. Schilke R, Bauss O, Lisson JA, Schuckar M & Geurtsen W (1999) Bovine dentin as a substitute for human dentin in shear bond strength measurements *American Journal of Dentistry* **12(2)** 92-96.
21. Nakamichi I, Iwaku M & Fusayama T (1983) Bovine teeth as possible substitutes in the adhesion test *Journal of Dental Research* **62(10)** 1076-1081.
22. Mellberg JR (1992) Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity *in situ Journal of Dental Research* **71 Special No** 913-919.
23. Jameson MW, Tidmarsh BG & Hood JA (1994) Effect of storage media on subsequent water-loss and regain by human and bovine dentine and on mechanical properties of human dentine *in vitro Archives of Oral Biology* **39(9)** 759-767.
24. Heyden P (1951) Studies on the moisture content of human teeth *Stomatologie* **4(2)** 77-97.
25. Papa J, Cain C & Messer HH (1994) Moisture-content of vital vs endodontically-treated teeth *Endodontics and Dental Traumatology* **10(2)** 91-93.
26. Helfer AR, Melnick S & Schilder H (1972) Determination of the moisture content of vital and pulpless teeth *Oral Surgery* **34(4)** 661-670.
27. Holcomb DW & Young RA (1980) Thermal decomposition of human tooth enamel *Calcified Tissue International* **31(3)** 189-201.
28. Holager J (1972) Thermogravimetric experiments on tooth carbonates *Journal of Dental Research* **51(1)** 102-106.
29. Fischer D & Bailey JH (1995) Vital bleaching for aesthetic improvement *Practical Periodontics and Aesthetic Dentistry* **7(8)** 61-64.
30. Seghi RR & Denry I (1992) Effects of external bleaching on indentation and abrasion characteristics of human enamel *in vitro Journal of Dental Research* **71(6)** 1340-1344.
31. Attin T, Muller T, Patyk A & Lennon ÁM (2004) Influence of different bleaching systems on fracture toughness and hardness of enamel *Operative Dentistry* **29(2)** 188-195.
32. Gosselin R, Smith R & Hodge H (1984) *Clinical Toxicology of Commercial Products* Williams and Wilkins, Baltimore 38, 73, 611, 704, 1627-1631.
33. Pashley DH & Carvalho RM (1997) Dentine permeability and dentine adhesion *Journal of Dentistry* **25(5)** 355-372.
34. Matthews WG, Showman CD & Pashley DH (1993) Air blast-induced evaporative water loss from human dentine, *in vitro Archives of Oral Biology* **38(6)** 517-523.