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Clinical Research

Spectroradiometric and Chemical Analysis of Severely Discolored Endodontically Treated Teeth

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Clinical Relevance

Discolored root-filled teeth are more sensitive to acid bleaching agents than normal natural teeth. Therefore, dentists must take precautions when bleaching root-filled teeth.

SUMMARY

Objective: This study aimed to explore the chemical changes and discoloration of endodontically treated teeth using spectroradiometric and chemical analyses.

Methods and Materials: Ten freshly extracted sound third molars and 10 discolored molars obtained after root canal treatment (RCT) were included in this study. Dentin blocks (3 $\times$ 3 $\times$ 1 mm) were prepared and treated with an intracoronal bleaching agent. Spectroradiometric evaluations, X-ray photoelectron spectroscopy (XPS), and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) analyses were performed before and after bleaching. The spectroradiometric parameters and XPS and ATR-FTIR spectra of the dentin slabs were analyzed using independent-sample *t*-tests or one-way analysis of variance.

Results: Differences in color coordinates and translucency parameters were observed between the two groups before bleaching. Demineralization effects were also detected in the discolored dentin. No binding energy shifts or new spectral bands were found in the XPS and ATR-FTIR spectra, except in the narrow-scanned XPS spectra of C_{1s}. Significant changes in color coordinates were also found in both groups after bleaching, and the translucency parameter was remarkably altered in discolored dentin. The organic and inorganic components of dentin also decreased in both groups. However, binding energy shifts (ie,

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DOI: 10.2341/15-243-C

C_{1s}, Ca_{2p}, and P_{2p}) were detected only in discolored dentin.

Conclusion: Significant alterations in the organic and inorganic components of dentin contributed to the discoloration of endodontically treated teeth. Moreover, intracoronal bleaching could effectively whiten both discolored and vital dentin. The chemical compositions of mineral and organic components within discolored dentin are more sensitive to the acid bleaching agent than normal teeth.

INTRODUCTION

Dental hard tissue discoloration frequently occurs after root canal treatment (RCT).[1-3] RCT-related tooth discoloration is usually mild and thus easily ignored by both patients and dentists. However, progressive and severe discoloration can lead to esthetic issues.[4,5] The appearance of teeth, especially that of the anterior teeth, is increasingly becoming of particular cosmetic importance to the public and among dental practitioners.[6,7] However, the discoloration mechanism of an endodontically treated tooth has not yet been fully clarified.

Tooth color is determined by interactions between the optical properties of dental tissues and light.[8-10] Any change in the structures and/or chemical compositions of teeth will likely alter its appearance.[11,12] Changes in endodontic materials, which can alter the structure and/or chemical compositions of dental tissues, are among the most common reasons for tooth discoloration after endodontic treatment.[12,13] Endodontic irrigation materials, such as chelators and sodium hypochlorite, can change the proportion of the organic and inorganic components of dentin.[14,15] Endodontic sealers, such as AH 26 and Kerr Pulp Canal Sealer, can also alter the chemical compositions of dentin by permeating into dentinal tubules.[16,17] Removal of pulp and the subsequent loss of moisture within dental tissues also affects the optical properties of teeth,[18] which is another possible reason for tooth discoloration after RCT. However, no direct chemical evidence explaining the effect of compositional changes in root-filled teeth on tooth color is available.

This study aims to explore the possible relationship between chemical changes in endodontically treated teeth and discoloration using spectroradiometric evaluation, X-ray photoelectron spectroscopy (XPS), and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Intracoronal bleaching was used as a positive treatment to induce potential chemical discrepancies between vital and severely discolored nonvital teeth. Our null hypothesis was that vital and severely discolored teeth are not significantly different in terms of optical properties or chemical composition.

METHODS AND MATERIALS

Tooth Selection

Ten vital third molars, which were extracted because of impaction or other orthodontic reasons, were included in this study and served as the normal color group (group NC). All of the teeth were free from stains, caries, or other defects. Tetracycline-stained teeth or teeth with fluorosis were excluded from this study.

Ten discolored molars that had been endodontically treated in the endodontics department of the Hospital of Stomatology, Wuhan University, were extracted for periodontal reasons and included in the study (group DC). The inclusion criteria for group DC were as follows: history of RCT (more than two years); composite fillings; absence of cracks and secondary caries; no use of chlorhexidine; no history of orthodontic intervention, prosthetic therapy, and bleaching; and no occupational exposure to metallic salts. The teeth were carefully examined after their surfaces were thoroughly cleaned, and those exhibiting extrinsic stains were excluded. The selected teeth were observed once more under a stereo microscope (Zeiss Stemi SV 11 Apo, Göttingen, Germany) after sectioning, and samples exhibiting obvious stains from the outer enamel extending into the inner dentin were also excluded.

The color of the extracted tooth and that of the neighboring teeth was measured using a commercial colorimeter (ShadeEye NCC, Shofu, Japan). Color differences (ΔE) between the extracted and neighboring teeth were calculated by equation 1: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$,[19] where ΔL^*, Δa^*, and Δb^* represent differences in lightness, green-red coordinates, and blue-yellow coordinates, respectively. The means of ΔE values for groups NC and DC were 2.12 ± 0.89 and 5.87 ± 2.36, respectively. All of the extracted teeth were cleaned thoroughly and stored in distilled water at 4°C until use.

Specimen Preparation

The teeth were longitudinally sectioned in the mesiodistal direction using a low-speed rotary diamond cutting instrument (SP1600, Leica Microsystems GmbH, Wetzlar, Germany) under water cooling. Two dentin blocks ($3 \times 3 \times 1$ mm) were

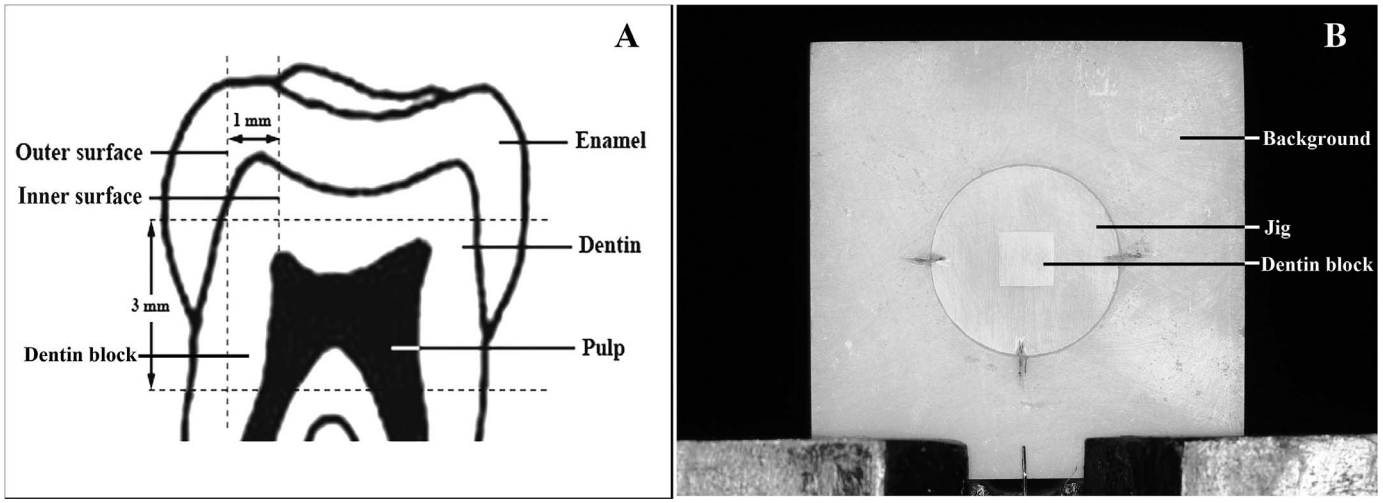

Figure 1. *(A): Schematic diagram of specimen fabrication. (B): Positioning system for color measurements.*

obtained from each tooth (Figure 1A). All the blocks were embedded in translucent acrylic resin, while both their inner surface (approaching the pulp cavity) and their outer surface (approaching the enamel) were left exposed. In addition, the inner and outer dentin surfaces were flattened using 800-, 1000-, 2000-, and 2500-grit SiC papers in sequence under continuous water cooling and then polished using 0.5 μm of aluminum oxide slurry. Finally, 1.0-mm-thick dentin slabs were achieved. The specimens were ultrasonically cleaned for 15 minutes and then stored in distilled water before testing.

An intracoronal bleaching gel containing 35% hydrogen peroxide (Opalescence Endo, Ultradent Product Inc, South Jordan, UT, USA) was applied to the inner surface of all dentin slabs under 100% humidity at 37°C according to the manufacturer's instructions. The bleaching gel was replaced every three days and applied twice.

Spectroradiometric Evaluations

One dentin slab of each tooth (10 dentin slabs in each group) was selected for color evaluation. A spectroradiometer (PR-655 Spectra Scan, Photo Research Inc, Chatsworth, CA, USA) equipped with Macro-Spectra MS-75 and SL-0.5X lenses was used for color measurements before and after bleaching. The standardized illuminant D65 (OL 53, Optronic Laboratories Inc, Orlando, FL, USA) at 2-degree observer and 0-/45-degree optical configurations were adopted in the color measurements.[20] The spectroradiometer was standardized to 91.4 mm from the measured object and a measurement aperture size of 1.5 mm in diameter. A customized jig with A3 shade background (Filtek Z350, 3M ESPE, St Paul, MN, USA) was used to fix the dentin slabs and confirm the position for repeated measurements (Figure 1B). To avoid dentin slab dehydration, we obtained each measurement within 10 seconds. The spectral reflectance of the specimens was also obtained at 380 to 780 nm at 2-nm intervals and then subsequently converted into CIE L*a*b* values.[21] ΔE values after bleaching were determined by equation 1.

The color coordinates of each specimen were measured against a white (CIE L*=99.99, a*=0.16, and b*=–0.03) and a black (CIE L*=2.24, a*=0.47, and b*=0.53) background before and after bleaching. The translucency parameter (TP) was calculated using equation 2: $TP = [(L_W^* - L_B^*)^2 + (a_W^* - a_B^*)^2 + (b_W^* - b_B^*)^2]^{1/2}$,[19] where the subscripts W and B refer to the color coordinates recorded against the white and the black background, respectively.

XPS Measurement

Five dentin slabs were randomly selected from each group for XPS evaluations before and after bleaching. The specimens were dried, evacuated at 107 Pa, and then introduced into a monochromatic X-ray photoelectron spectrometer (ESCALAB 250Xi, Thermo Scientific Inc, Waltham, MA, USA) equipped with an Al Kα X-ray radiation source. The photoelectrons emitted from the Al Kα X-ray radiation source (1486.6 eV) were analyzed using a 180-degree hemispherical capacitor electron energy analyzer. The XPS survey spectra were obtained at 0 to 1350.21 eV with a pass energy of 150 eV at a step

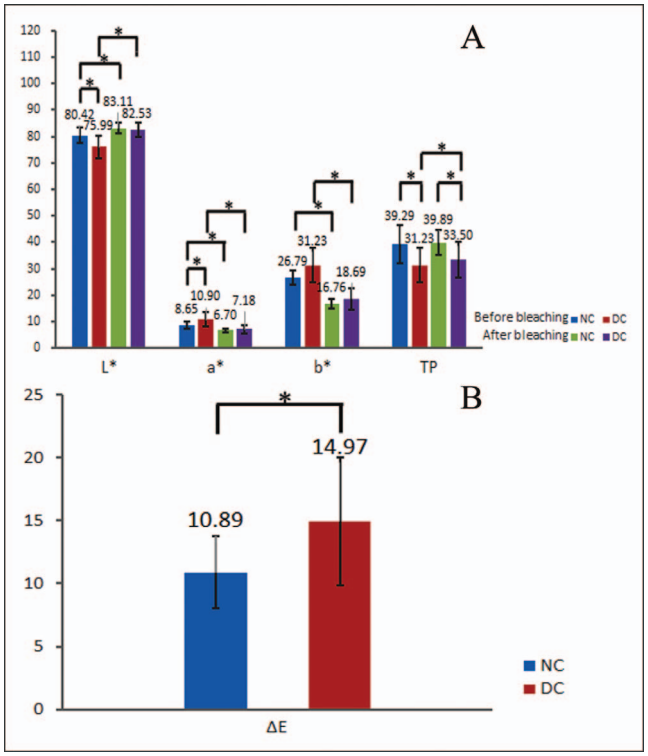

Figure 2. *Means of color coordinates (L*, a*, and b*), translucency parameter (A), and ΔE values (B) of both groups before and after bleaching. (superscript * in the figure denotes significant difference between two groups;* $p<0.05$*).*

of 1.0 eV. The narrow-scanned spectra were collected with a pass energy of 30 eV at a step of 0.05 eV. The binding energy scales for the specimens were calibrated by setting the value of the carbon bonded to either hydrogen or carbon (C–H/C–C) as a reference at 284.80 eV. The spectral data were analyzed by Avantage Software (Thermo Scientific), the averaged atomic concentrations of C_{1s}, O_{1s}, Ca_{2p}, and P_{2p} were recorded, and the Ca/C, P/C, and Ca/P ratios were calculated.

ATR-FTIR Measurement

The remaining five dentin slabs in each group were analyzed using an FTIR spectroradiometer (Nicolet Impact 420, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a diamond crystal ATR unit. The spectra were collected at 800 to 1600 cm^{-1} at a resolution of 4 cm^{-1} to achieve a total of 64 scans and then analyzed by OMNIC 8 software (Nicolet, Madison, WI, USA). The phosphate/amide ratio (the ratio of the integrated areas of phosphate υ_1, υ_3 to that of amide I peak) of ATR spectra were examined before and after bleaching.[22]

Statistical Analysis

Differences in color coordinates (L*, a*, and b*), TP, and phosphate/amide ratios between the two groups were analyzed using independent-sample *t* tests. Analysis of variance was performed to evaluate differences in the effects of bleaching between the ΔTP and ΔE values of the two groups. All analyses were accomplished using SPSS for Windows (SPSS, Chicago, IL, USA), and the level of significance was established at $\alpha = 0.05$.

RESULTS

Spectroradiometric Evaluations

Figure 2A shows the means of the color coordinates (L*, a*, and b*) and TP values. The L* and a* values of the two groups were significantly different before bleaching. Moreover, the TP values were significantly lower in group DC than in group NC both before and after bleaching ($p<0.01$). Bleaching did not significantly influence the TP values of group NC but remarkably increased those of group DC ($p<0.05$). In addition, the color coordinates (L*, a*, and b*) of both groups were significantly changed after bleaching, and the ΔE value was significantly greater in group DC than in group NC ($p<0.05$; Figure 2B).

XPS Analysis

Figure 3 shows the representative wide-scanned XPS spectra of the inner dentin surfaces before and after bleaching. The heights of C_{1s}, Ca_{2p}, and P_{2p} peaks in both groups were lower after bleaching than before bleaching. Figure 4A through F shows the narrow-scanned XPS spectra and the binding energies of the elements Ca_{2p} and P_{2p}. The spectral profiles and binding energies of these elements in the two groups were similar before bleaching. However, the binding energies of C_{1s}, Ca_{2p}, and P_{2p} in group DC obviously shifted toward higher energies compared with those in group NC after bleaching.

A low protrusion was observed in the narrow-scanned XPS spectra of C_{1s} of both groups before bleaching (Figure 4A). The peak at 288.13 eV in group NC corresponds to the carbon double bonded to oxygen (C=O), which is most probably related to either the carboxyl groups (COOH) or the amide bond (peptide bond) (CONH) of dentin proteins. However, the peak of the low protrusion in group DC shifted to 287.93 eV, possibly indicating deoxidization of C=O. After bleaching, the low protrusion in the narrow-scanned XPS spectra of C_{1s} nearly disappeared in both groups (Figure 4D).

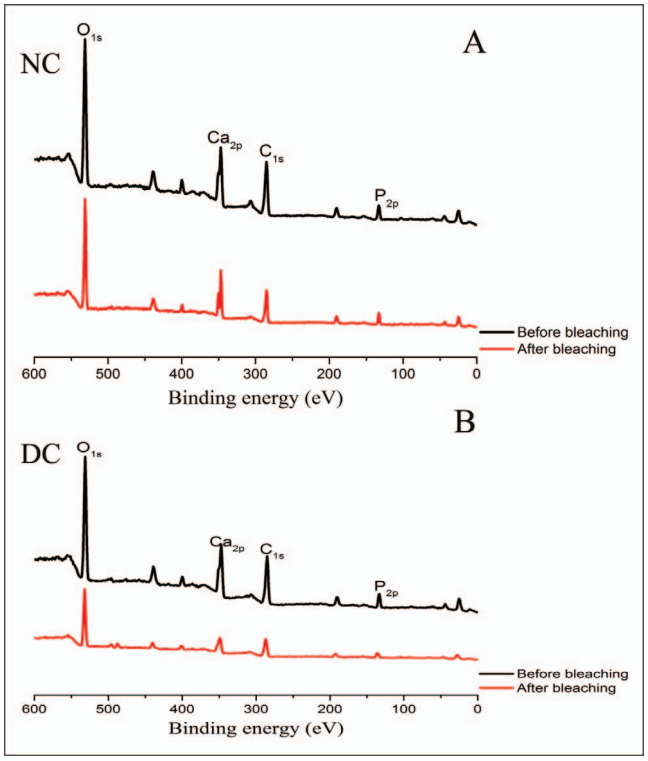

Figure 3. *Wide-scanned X-ray photoelectron spectroscopy spectra before and after treatment. (A) Group NC. (B) Group DC.*

Curve fitting was used in analyzing the components of the C_{1s} region in group DC because of the remarkable change in its peak profile and binding energy after bleaching (Figure 4G). The C_{1s} spectrum of the discolored dentin was fitted with three components at binding energies of 284.83, 286.83, and 289.73 eV, respectively. The peak at 284.83 eV corresponds to aliphatic hydrocarbons [C–(C,H)], which are related to the organic components, whereas the peak at 286.83 eV includes single carbon bonded to either oxygen or nitrogen [C–(O,N)], which may originate from proteins. The component at 289.73 eV corresponds to the carbonate groups (CO_3^{2-}) present in the mineral component of dentin.

The relative atomic ratios of the dentin slabs were also calculated before and after bleaching (Figure 4H). Before bleaching, the Ca/C, P/C, and Ca/P ratios were lower in the inner discolored dentin surface than in the vital one ($p<0.05$). After bleaching, the Ca/P remained significantly lower in group DC than in group NC ($p<0.05$). Bleaching treatment can thus greatly influence the Ca/C and P/C ratios of both groups ($p<0.05$).

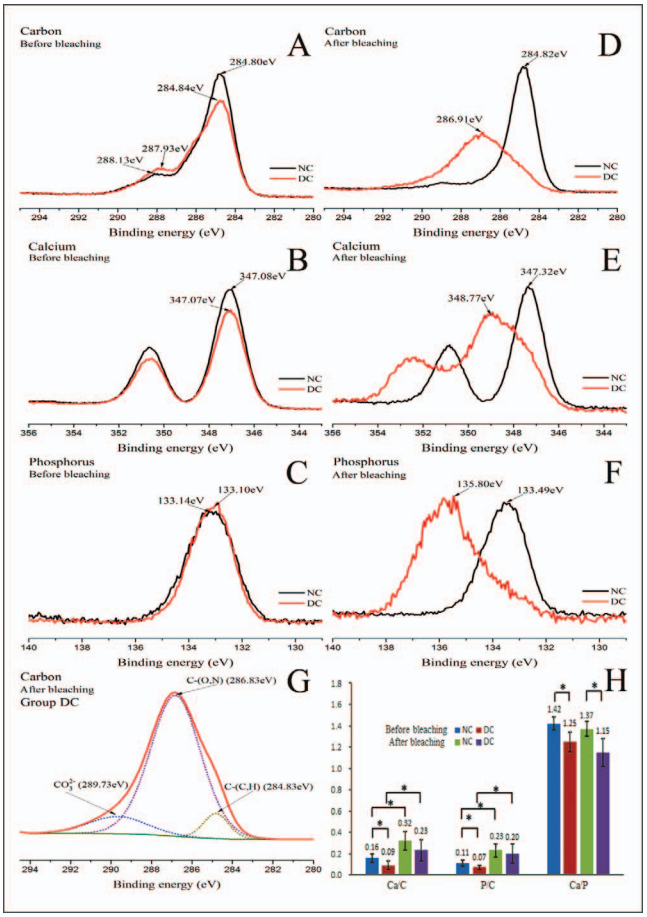

Figure 4. *Narrow-scanned X-ray photoelectron spectroscopy spectra of elements (C_{1s}, Ca_{2p} and P_{2p}) of both groups before bleaching (A-C) and after bleaching (D-F). Curve fitting of the C_{1s} region in group DC after bleaching (G). Means of Ca/C, P/C, and Ca/P ratio of both groups before and after bleaching. (H) (superscript * in the figure denotes significant difference between two groups; p<0.05).*

ATR-FTIR Analysis

Figure 5 shows the representative ATR-FTIR spectra of the inner dentin surfaces before and after bleaching. The characteristic absorption bands of the collagen matrix and the hydroxyapatite mineral components were identified and marked in the spectra according to the literature.[23-25] The ATR spectra showed that the relative intensities of phosphate υ_1,υ_3 were significantly lower in group DC than in group NC before bleaching (Figure 5A). Moreover, the relative intensities of phosphate υ_1,υ_3, carbonate υ_2, and amides I, II, and III decreased in both groups after bleaching (Figure 5B). More dramatic decreases in the relative intensities of these components were also detected in group DC. The phosphate/amide ratio was significantly lower in group DC than in group NC before and after bleaching ($p<0.05$; Figure 5C).

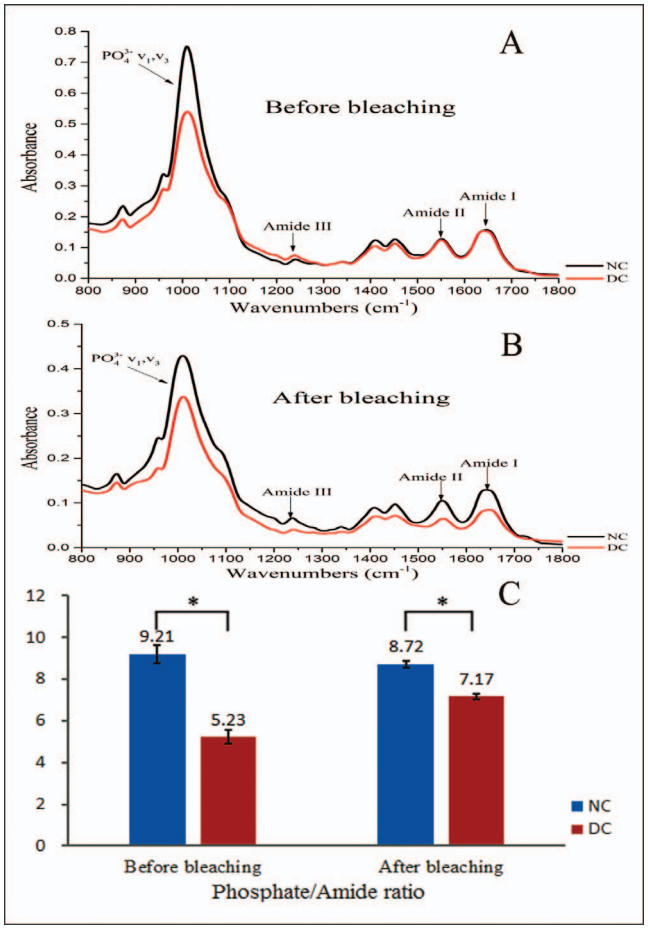

Figure 5. *Attenuated total reflection spectra of both groups before bleaching (A) and after bleaching (B). Means of phosphate/amide ratio of both groups before and after bleaching (C). (superscript * in the figure denote significant difference between two groups (* $p<0.05$).*

DISCUSSION

Our null hypothesis was rejected because the chemical compositions and optical properties of the discolored dentin were evidently different from those of the vital dentin both before and after bleaching.

Mountouris[26] and Hülsmann[27] reported that the chemicals used in endodontic irrigation and/or disinfection potentially affect the mineral and organic contents of dentin. Chelators, such as EDTA, are widely used in root canal irrigation, and they interact with the calcium in dentin by forming a stable complex with calcium, leading to decalcification.[28,29] The ATR spectra revealed that the relative intensities of phosphate υ_1,υ_3 were obviously lower in the discolored dentin than in the vital dentin (Figure 5A). Moreover, the phosphate/amide ratio was significantly lower in the discolored dentin than in the vital dentin. XPS analysis further showed the significant decrease in the atomic ratio of Ca/C, P/C, and Ca/P in the discolored dentin. These results reveal that the amount of mineral components was remarkably lower in the discolored dentin, indicating that dentin demineralization occurred in discolored dentin after RCT.

Chromatic alteration is the most noticeable change in nonvital teeth after RCT. The spectroradiometric evaluations demonstrated significant differences in the color coordinates (L*, a*, and b*) and TP values of the two groups. Light propagation through human dentin was affected by dentinal tubules and collagen fibers in the dentin.[8,30] Any change in the organic and inorganic components of dentin may affect the light transmission through the dentinal tubules, which will greatly influence the optical properties of dentin. In terms of the narrow-scanned XPS spectra of C_{1s} before bleaching, a low protrusion occurred at approximately 288.13 eV in normal dentin and at approximately 287.93 eV in discolored dentin. Moreover, the peak heights at 288.84 and 287.93 eV were lower and higher, respectively, in discolored dentin than in normal dentin. These results indicate that aliphatic hydrocarbons [C–(C,H)] were decomposed and that new chemical groups were formed in the organic components of discolored dentin. Grossman[31] proposed that pulpal necrosis and pulpal hemorrhage contribute to teeth discoloration. This supposition was subsequently modified by Ingle,[32] who considered that discoloration also frequently results from necrotic pulp tissue decomposition even though frank hemorrhage has not occurred. However, whether the blood components of the pulp tissue, the necrotic pulp tissue, or both are the primary causes of tooth discoloration has not yet been understood. New chemical groups formed in the organic components of discolored dentin most likely originated from hemolysis of red blood cells and/or decomposition of necrotic pulp tissue. The organic compositions of discolored dentin were exposed after dentin demineralization and bonded to the new chemical groups, thereby forming the chromophores and causing tooth discoloration.

To explore the possible mechanism of color change in root-filled teeth, a positive treatment of intracoronal bleaching was performed to induce potential variations between the two groups. The most noticeable change in group DC after bleaching is the shifts in its binding energies of C_{1s}, Ca_{2p}, and P_{2p} toward higher energies. This phenomenon indicates that the chemical structure of the mineral and organic components of discolored dentin were significantly influenced by bleaching. Moreover, the

reduced Ca/P ratio in both groups implies demineralization of dentin. The wide-scan XPS spectra of both groups demonstrated a decline in the peak height of Ca_{2p} and P_{2p} after bleaching. This demineralization effect can be explained by the low pH of Opalescence Endo (pH = 3.0 to 5.0).[22,33,34] The Ca/P ratio remained significantly lower in discolored dentin than in vital dentin after bleaching. The ATR spectrum also showed that the relative intensities of phosphate υ_1, υ_3 were obviously lower in the discolored dentin than in the vital dentin after bleaching. The binding energies of Ca_{2p} and P_{2p} in the discolored dentin surface shifted toward higher energies after bleaching. These results suggest that the mineral substance (hydroxyapatite) of discolored dentin suffers more damage after bleaching and that its chemical structure is altered by the strong oxidation property of the intracoronal bleaching agent.

With respect to the change in organic components of the discolored dentin, curve fitting of the narrow-scan spectrum of the C_{1s} region revealed that the primary component of the carbon-containing compounds in the discolored dentin surface after bleaching was the carbon single bonded to oxygen (C–O) instead of hydrocarbons (C–C/C–H/C=C). This phenomenon can be explained by the oxidation effect of the intracoronal bleaching agent on the organic content of the discolored dentin.[35-37] Being the active ingredient of the intracoronal bleaching agent, hydrogen peroxide can break down and produce free radicals, such as hydroxyl radicals, perhydroxyl radicals, perhydroxyl anions, and superoxide anions.[37] By cleaving the conjugated chain or by oxidizing other chemical moieties in the conjugated chain, the reactive molecules, which often include heteroatoms, carbonyl, and phenyl rings in the conjugated system, can decompose the chromophores in discolored dentin.[38] Compared with those of vital dentin, the organic components of the discolored dentin suffer more serious damage after bleaching. The ATR spectrum showed that the relative intensities of amides were obviously lower in the discolored dentin than in the vital one. Moreover, the phosphate/amide ratio significantly increased in discolored dentin after bleaching. During bleaching, the surface organic contents of dentin are quickly removed by the bleaching agent; the surface mineral components also collapse and then form a protective layer to reduce the destruction of the underlying dentin.[22] Therefore, the discolored dentin is more sensitive to bleaching agents due to the low density of mineral contents on its surface.

The color coordinates (L*, a*, and b*) and the TP value of the discolored dentin were greatly influenced by bleaching. Dentinal tubules are the main cause of light scattering in dentin.[38] When exposed to 30% hydrogen peroxide, the organic components of the dentinal tubules, including chromophores and collagen fibers, were decomposed, as result of the strong oxidizing ability of hydrogen peroxide.[22,36] The intertubular and peritubular dentin were also dissolved and lost due to the acidity of hydrogen peroxide.[22,36] Such changes enlarged the orifices of the dentinal tubules and increased their permeability,[39] which might have reduced the light scattering and allowed more light to pass through the dentinal tubules. Thus, remarkable alterations in color coordinates and translucency of the discolored dentin were caused by greater amount of light reflected from the black, white, and A3 shade backgrounds. By contrast, the TP value of the vital dentin did not significantly change after bleaching. The ΔE in the vital dentin was significantly lower than that in discolored dentin. On the basis of the chemical analysis, we can safely conclude that the optical properties of dentin are closely associated with the chemical structure of the inorganic and organic substances within dentin. Moreover, significant changes in the color coordinates and translucency of the discolored dentin were caused by remarkable alterations in the chemical structure of the mineral and organic components within the discolored dentin.

The etiology of the discoloration of endodontically treated teeth is the most important issue in the analyses. This study excluded the extrinsic causes of discoloration, and thus strict inclusion criteria were set during specimen selection to ensure that only intrinsic factors were considered. However, some of the criteria may be extracted mainly from the medical history and/or patients' memory, and possible errors may influence the results. To obtain accurate and repeatable measurements, the inner dentin surface was flattened during specimen preparation. Removal of the inner region of the dentin may have altered its chemical compositions. Further studies are thus necessary to clarify these limitations.

CONCLUSIONS

Within the limitations of this study, the following conclusions were drawn: 1) the optical properties of dentin are closely associated with the chemical structures of inorganic and organic substances with significant changes in the proportion of organic and

inorganic components of dentin contributing to the discoloration of root-filled teeth; 2) intracoronal bleaching can effectively whiten both discolored nonvital and vital dentin although the chemical structure of the mineral and organic components of the discolored dentin may suffer more serious bleaching-induced damages.

Acknowledgements

The study was supported by grants from the Hubei Province Health and Family Planning Scientific Research Project (No. WJ2015Q025) and Natural Science Foundation of Hubei Province (No. 2013CFA068).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee of the School and Hospital of Stomatology, Wuhan University. The approval code for this study is 2014.9.1.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 17 December 2015)

REFERENCES

1. Gutiérrez JH, & Guzmán M (1968) Tooth discoloration in endodontic procedures *Oral Surgery, Oral Medicine, Oral Pathology* **26(5)** 706-711.
2. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, & Krastl G (2012) Tooth discoloration induced by endodontic materials: A laboratory study *International Endodontic Journal* **45(10)** 942-949.
3. Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, & Weiger R (2013) Tooth discoloration induced by endodontic materials: A literature review *Dental Traumatology* **29(1)** 2-7.
4. Abbott PV (1997) Aesthetic considerations in endodontics: Internal bleaching *Practical Periodontics and Aesthetic Dentistry* **9(7)** 833-840.
5. Davis MC, Walton RE, & Rivera EM (2002) Sealer distribution in coronal dentin. *Journal of Endodontics* **28(6)** 464-466.
6. Hattab FN, Qudeimat MA, & Al-Rimawi HS (1999) Dental discoloration: An overview *Journal of Esthetic and Restorative Dentistry* **11(6)** 291-310.
7. Sulieman M (2005) An overview of tooth discoloration: Extrinsic, intrinsic and internalized stains *Dental Update* **32(8)** 463.
8. Zijp JR, & ten Bosch JJ (1993) Theoretical model for the scattering of light by dentin and comparison with measurements *Applied Optics* **32(4)** 411-415.
9. Fried D, Glena RE, Featherstone JD, & Seka W (1995) Nature of light scattering in dental enamel and dentin at visible and near-infrared wavelengths *Applied Optics* **34(7)** 1278-1285.
10. Joiner A, Hopkinson I, Deng Y, & Westland S (2008) A review of tooth colour and whiteness *Journal of Dentistry* **36(Supplement 1)** S2-S7.
11. AM Sulieman M (2008) An overview of tooth-bleaching techniques: Chemistry, safety and efficacy *Periodontology 2000* **48(1)** 148-169.
12. Watts A, & Addy M (2001) Tooth discolouration and staining: A review of the literature *British Dental Journal* **190(6)** 309-316.
13. Dahl JE & Pallesen U (2003) Tooth bleaching—A critical review of the biological aspects *Critical Reviews in Oral Biology and Medicine* **14(4)** 292-304.
14. Hülsmann M, Heckendorff M, & Lennon A (2003) Chelating agents in root canal treatment: Mode of action and indications for their use *International Endodontic Journal* **36(12)** 810-830.
15. Mohammadi Z (2008) Sodium hypochlorite in endodontics: An update review *International Dental Journal* **58(6)** 329-341.
16. Çalt S, & Serper A (1999) Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide *Journal of Endodontics* **25(6)** 431-433.
17. Parsons JR, Walton RE, & Ricks-Williamson L (2001) In vitro longitudinal assessment of coronal discoloration from endodontic sealers *Journal of Endodontics* **27(11)** 699-702.
18. Salerno FR (1967) Bleaching the discolored tooth *Journal of the Wisconsin State Dental Society* **43(12)** 341.
19. Berns RS, Billmeyer FW Jr, & Saltzman M (2000) *Billmeyer and Saltzman's Principles of Color Technology* Wiley, New York, NY.
20. Bolt RA, ten Bosch JJ, & Coops JC (1994) Influence of window size in small-window colour measurement, particularly of teeth *Physics in Medicine and Biology* **39(7)** 1133-1142.
21. CIE (1978) Recommendations on uniform color spaces, color difference equations, psychometric color terms *CIE Publication* **(Supplement 2)** E-13-1.
22. Jiang T, Ma X, Wang Y, Zhu Z, Tong H, & Hu J (2007) Effects of hydrogen peroxide on human dentin structure *Journal of Dental Research* **86(11)** 1040-1045.
23. Barth A, & Zscherp C (2002) What vibrations tell about proteins *Quarterly Reviews of Biophysics* **35(04)** 369-430.
24. Arai Y, & Sparks DL (2001) ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface *Journal of Colloid and Interface Science* **241(2)** 317-326.
25. Antonakos A, Liarokapis E, & Leventouri T (2007) Micro-Raman and FTIR studies of synthetic and natural apatites *Biomaterials* **28(19)** 3043-3054.
26. Mountouris G, Silikas N, & Eliades G (2003) Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin *Journal of Adhesive Dentistry* **6(3)** 175-182.

27. Hülsmann M (2013) Effects of mechanical instrumentation and chemical irrigation on the root canal dentin and surrounding tissues *Endodontic Topics* **29(1)** 55-86.

28. Verdelis K, Ellades G, Ovllr T, & Margelos J (1999) Effect of chelating agents on the molecular composition and extent of decalcification at cervical, middle and apical root dentin locations *Dental Traumatology* **15(4)** 164-170.

29. Cobankara FK, Erdogan H, & Hamurcu M (2011) Effects of chelating agents on the mineral content of root canal dentin *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **112(6)** e149-e154.

30. Hariri I, Sadr A, Shimada Y, Tagami, J & Sumi Y (2012) Effects of structural orientation of enamel and dentine on light attenuation and local refractive index: An optical coherence tomography study *Journal of Dentistry* **40(5)** 387-396.

31. Grossman L (1943) *Root Canal Therapy* **2nd edition** Lea & Febiger, Philadelphia, PA.

32. Ingle JI (1974) *Endodontics* Lea & Febiger, Philadelphia, PA.

33. Carrasco-Guerisoli LD, Schiavoni RJDS, Barroso JM, Guerisoli DMZ, Pécora JD, & Fröner IC (2009) Effect of different bleaching systems on the ultrastructure of bovine dentin *Dental Traumatology* **25(2)** 176-180.

34. Sulieman M, Addy M, Macdonald E, & Rees JS (2004) A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentine *Journal of Dentistry* **32(7)** 581-590.

35. Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, & Tamimi F (2012) Hydrogen peroxide whitens teeth by oxidizing the organic structure *Journal of Dentistry* **40(Supplement 2)** e25-e33.

36. Kawamoto K, & Tsujimoto Y (2004) Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching *Journal of Endodontics* **30(1)** 45-50.

37. Gregus Z, & Klaassen CD (1995) Mechanisms of toxicity In: Klaassen CD (ed) *Cassarett and Doull's Toxicology, the Basic Science of Poisons* McGraw-Hill, New York, NY 35-74.

38. Howe-Grant M (1992) *Encyclopedia of Chemical Technology* John Wiley and Sons, New York, NY.

39. Vaarkamp J, Ten Bosch JJ, & Verdonschot EH (1995) Propagation of light through human dental enamel and dentine *Caries Research* **29(1)** 8-13.

40. Heling I, Parson A, & Rotstein I (1995) Effect of bleaching agents on dentin permeability to *Streptococcus faecalis* *Journal of Endodontics* **21(11)** 540-542.