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Effects of Different Concentrations and Exposure Time of Sodium Hypochlorite on the Structural, Compositional and Mechanical Properties of Human Dentin*

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Summary: This study evaluated the effects of sodium hypochlorite (NaOCl) with different concentrations and exposure time on the structural, compositional and mechanical properties of human dentin *in vitro*. Sixty dentin slabs were obtained from freshly extracted premolars, randomly distributed into four groups (n=15), and treated with 1%, 5%, 10% NaOCl and distilled water (control group), respectively, for a total of 60 min. Attenuated total reflection infrared (ATR-IR) spectroscopy, Raman spectroscopy and X-ray diffraction (XRD) were carried out before, 10 min and 60 min after the treatment. Scanning electron microscopy (SEM) and flexural strength test were conducted as well. The results showed that dentins experienced morphological alterations in the NaOCl groups, but not in the control group. Two-way repeated-measures analysis of variance revealed that the carbonate:mineral ratio (C:M), Raman relative intensity (RRI), a-axis, c-axis length and full width at half maximum (FWHM) with the increase of time and concentration in the NaOCl groups were not significantly different from those in the control group (P>0.05). Nevertheless, the mineral:matrix ratio (M:M) increased and the flexural strength declined with the increase of concentration and the extension of time in the NaOCl groups (P<0.05). Additionally, it was found that the M:M and the flexural strength remained unchanged after 1% NaOCl treatment (P>0.05), and the morphology changes were unnoticeable within 10 min in 1% NaOCl group. These results indicated that NaOCl has no significant effects on the inorganic mineral of human dentin; but it undermines and eliminates the organic content concentration- and time-dependently, which in turn influences the flexural strength and toughness of dentins. In addition, an irrigation of 1% NaOCl within 10 min can minimize the effects of NaOCl on the structural and mechanical properties of dentin during root canal treatment.
Key words: sodium hypochlorite; dentin; attenuated total refletion infrared spectroscopy; Raman spectroscopy; scanning electron microscopy; flexural strength

Dentin, the most abundant part of dental hard tissue, consists of organic matric embedded in crystalline apatites[1]. Such pattern of mineralized collagen fibril constructs a topological continuous network, which greatly contributes to the mechanical characteristics of dentin[2]. The correlation between the dentinal structure and its mechanical properties has been extensively studied[3], especially after dentists noticed that some dental treatments might result in changes of the related manifestations in clinical practice.

Root canal treatment (RCT) is a common endodontic therapy, which removes the inflamed or necrotic pulp and the infected dentin. During the process of RCT, an effective cleansing of the root canal system is necessary, and the root canal irrigation plays a pivotal role in the whole procedure[4, 5]. Among the multiple irrigation solutions, sodium hypochlorite (NaOCl) is the most widely used due to its wide-spectrum antimicrobial efficacy[6, 7] and distinctive capacity to dissolve necrotic pulp tissue remnants[8, 9]. Additionally, with the nonspecific oxidizing and proteolytic ability, NaOCl oxidizes and denatures the collagen content of the smear layer from root canals[10, 11].

Although there is little doubt about its antimicrobial and necrotic tissue-dissolving capacity, controversy remains about the impact of NaOCl with different concentrations or exposure time on dentin structure. Sauro *et al*[12] discovered that 12% (w/v) NaOCl could not achieve a complete deproteinization of acid-etched dentin in a maximum clinically possible period of 120 s; whereas Marshall *et al*[13] found that 6.5% (w/v) NaOCl removed much of the dentinal collagen after 120 s. Zhang *et al*[14, 15] suggested that the removal of the organic phase from the mineralized dentin by NaOCl was both concentration- and time-dependent. However, Hu *et al*[16] indicated the different exposure time of NaOCl with the same concentration did not influence its effect of deproteinization on dentin.

The inconsistent conclusions, we believe, are primarily attributed to different study designs and methods. As we know, diverse detection techniques lead to differ-

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ent results. Previous studies were mainly conducted using the invasive detection techniques, such as Fourier transform infrared (FTIR) spectroscopy, thermal gravimetric analysis (TGA), etc. Samples detected by these methods could not act as their own controls to be measured repeatedly, which might result in bias. Therefore, non-invasive techniques collecting data in real time are needed to evaluate the effects of NaOCl with different concentrations or exposure time on the dentin structure.

With the advances in technologies and interdisciplinary knowledge, a number of noninvasive and powerful techniques have been used in the field of dentistry and have provided new insights into researches about dentinal structure. One of our previous studies[17] noted that the attenuated total reflection infrared (ATR-IR) spectroscopy could exceed FTIR in detecting the minor variations of dentinal structure at the molecular level after bleaching treatments. ATR-IR spectroscopy permits repeated analyses of the sample surface at the same site, which ensures high comparability between spectra before and after treatment. In contrast, for conventional FTIR, samples need to be scraped off and ground into fine powders. Any less-affected subsurface regions could mask the small surface structural changes. Raman spectroscopic technique is another spectroscopic technique that can analyze the chemical structure of dental specimens at the molecular level non-invasively[18]. Previous studies[19] successfully proved that Raman spectroscopy could sensitively detect the mineral changes through capturing the alteration of particular chemical bands, *i.e.* $v_1PO_4^{-3}$ at 960 cm^{-1}. Furthermore, laser-induced fluorescence, which was usually regarded as the interference of Raman scattering, can be measured and may even provide important information about the organic matter in dentin[20].

The purpose of this study was to evaluate the effects of different concentrations and exposure time of NaOCl on the human dentinal structure, mainly based on the evidence provided by the real-time ATR-IR and Raman spectroscopy. To better understand the spectroscopic results, X-ray diffraction (XRD), scanning electron microscopy (SEM) and flexural strength test were added as complementary means to further reveal how the structural and compositional changes affect the morphological and mechanical properties of dentin.

1 MATERIALS AND METHODS

1.1 Tooth Selection

The study protocol was reviewed and approved by the Ethics Committee of the School and Hospital of Stomatology, Wuhan University, China. Sixty fresh intact human premolars were obtained with informed consent from orthodontically treated patients. All the teeth were cleaned thoroughly and stored in 0.5% thymol at 4°C until use.

1.2 Specimen Preparation

The roots of teeth were separated from their crowns at the cement-enamel junction using a low speed diamond saw (Isomet, Buehler Ltd., USA) under water cooling and a dental slab was obtained from each tooth. The surrounding enamel was cut off, creating the cuboid dentin specimen (4 mm×3 mm×2 mm). Each specimen was then fixed in polyvinyl chloride matrix with acrylic resin matrix, keeping the targeted surface unsealed for NaOCl treatments. To ensure the precision of all measurements, unsealed surfaces were prepared to be uniform, flat, and clean. Specimens were serially polished with 1000-, 2000-, 3000- and 5000-silicon carbide abrasive papers and with 1-μm and 0.5-μm diamond polishing suspensions on cloths under constant water irrigation. Finally, the specimens were immersed in distilled water (DW) and ultrasonically cleansed for 5 min to remove the residual particles and the smear layer.

1.3 Treatment Procedure

Specimens were divided randomly into four groups: DW group, 1% NaOCl group, 5% NaOCl group and 10% NaOCl group. Each specimen was immersed into 2 mL DW or NaOCl solution (Sinopharm Chemical Reagent Co. Ltd., China) and the total treatment time was set as 60 min. All experiments were carried out at room temperature, *i.e.* around 22°C.

1.4 ATR-IR Spectroscopy Detection

Three specimens in each group were subjected to ATR-IR spectroscopy detections and reference points were marked for each specimen to perform the analysis at the same positions before and after treatment. ATR-IR spectra were obtained with a Thermo Nicolet 5700 spectrometer (Nicolet, USA) equipped with a diamond crystal accessory as an internal reflection element. Each spectrum was collected in the range from 800 to 1800 cm^{-1} at the resolution of 4 cm^{-1} with 128 scans co-added at room temperature. Each specimen was measured at three different sites for an average value. The obtained ATR-IR spectra were analyzed using OMNIC 7 software (Nicolet, USA). After water subtraction, baseline correction and normalization, the mineral:matrix (the ratio of the integrated areas of $v_1v_3PO_4^{-3}$ contour to amide I peak, M:M) and the carbonate:mineral (the ratio of the integrated areas of $v_2CO_3^{-2}$ contour to $v_1v_3PO_4^{-3}$ contour, C:M) before and after treatment were calculated[17].

1.5 Raman Spectroscopy Detection

The same three specimens in each group were detected by Raman spectroscopy and reference points were marked for each specimen to perform the analysis at the same positions before and after treatment. Raman scattering/fluorescence spectra were recorded using a micro-Raman spectrometer (i-Raman Portable Raman Spectrometer, B&W TEK Inc., USA) equipped with a semiconductor laser diode (785 nm wavelength). A focused laser spot with approximately 95 μm size was shone on the surfaces of dentin through a fiber-optic based system. Each spectrum was made an average of five times in the range from 0 to 3200 cm^{-1} in the integration time of 7000 ms at room temperature.

Spectral data were visualized on a computer and processed using BWSpec 3.26 spectroscopic software (BWSpec, B&W TEK Inc., USA). Raman absolute intensity (RAI), Raman relative intensity (RRI), and laser-induced fluorescence intensity (FI) before and after treatment were defined and calculated according to a previous study[19]. RAI is the intensity of the Raman peak at 960 cm^{-1} before subtracting the baseline, and RRI is the intensity of the same peak after subtracting the baseline between 990 and 930 cm^{-1}. FI equals to RAI minus RRI (fig. 1). All values were transformed into percentage values where the baseline values were set at 100% and the values afterward were calculated as a percentage of

the baseline.

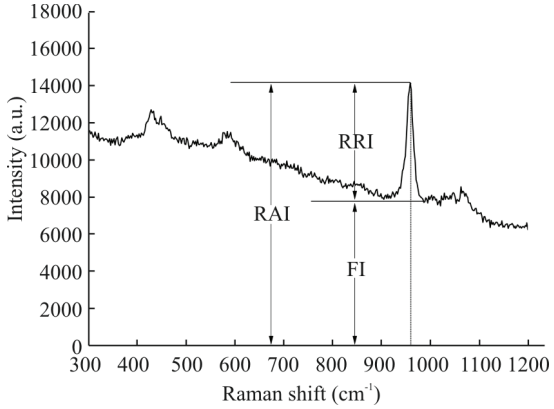

Fig. 1 Representative dentinal Raman/fluorescence spectrum RAI, RRI, and FI at 960 cm^{-1} are shown.

1.6 XRD Detection

The same three specimens in each group were tested for XRD using an X'pert PRO MPD (PANalytical, Almelo, Netherlands) with a Cu K_α generator working at 40 kV and 40 mA. Each spectrum was collected in the range of scanning angle from 20° to 60° with a wavelength of 1.5406Å in a continuous mode at the rate of 25 s per step and a step size of 0.02 (2θ) at room temperature. The XRD spectra were processed using JADE 6.5 (Materials Data, Inc., USA). The crystal phase, the full width at half maximum (FWHM) of (002) peak and the cell parameters (a-axis and c-axis length) before and after treatment were calculated.

1.7 SEM Detection

Another three specimens were removed from the acrylic resin matrix with probes for SEM in each group. These dentinal specimens were ultrasonically cleaned with DW, dried in a desiccator and coated with gold in a vacuum evaporator (JEOL JFC1600, Japan). The treated surfaces were observed using a field emission scanning electron microscope (Sigma, Germany) at 20 kV.

1.8 Three-point Loading Test

Another nine specimens were tested for flexural strength in each group (three for a time point). Before test, the slabs were sectioned into 36 plano-parallel dentine bars (0.8×0.8×> 11.7 mm). After treatment with varied solutions, the bars were placed across the lower supports of the test jig that was mounted on a loading test machine (Instron Ltd., USA) and then loaded at the mid-point through the loading head and shaft. All the dentine bars were kept moist with DW during testing. The loading test machine was run at a speed of 0.5 mm/min until failure. Data were recorded on a plotter to give load-displacement curves on graph paper and the load at fracture was recorded directly from the loading test machine. Flexural strength (MPa) was calculated according to the formula $3PL/2bd^2$, where L is the support span width (mm), b is the width (mm), d is the depth (mm) of the sample and P is the load (N) at fracture[21].

1.9 Statistical Analyses

Statistical analysis was performed using SPSS 19.0 for WINDOWS. Overall effects of treatment were analyzed with two-way repeated-measures analysis of variance (RMANOVA) (treatment time as the within-group factor and treatment concentration as the between-groups factor). After RMANOVA, the paired *t*-test was used to analyze the effect of treatment time and the *post hoc* Tukey test was used to analyze the effect of treatment concentrations. The level of significance was set at a *P* value of 0.05.

2 RESULTS

2.1 ATR-IR Spectrum Analysis

The characteristic ATR-IR spectra of dentin before and after treatment are displayed in fig. 2A–2D. The band between 885 and 1090 cm^{-1} gave information about $\nu_1\nu_3PO_4^{-3}$, between 810 and 885 cm^{-1} about $\nu_2CO_3^{-2}$, between 1600 and 1700 cm^{-1}, 1510 and 1580 cm^{-1}, 1220 and 1340 cm^{-1} about amide Ⅰ, amide Ⅱ and amide Ⅲ, respectively.

The contour of $\nu_1\nu_3PO_4^{-3}$ remained almost unchanged after treatment in all groups. The amide peaks were stable after treatment in the DW group (fig. 2A), while they gradually decreased with treatment time in the NaOCl groups (fig. 2B–2D).

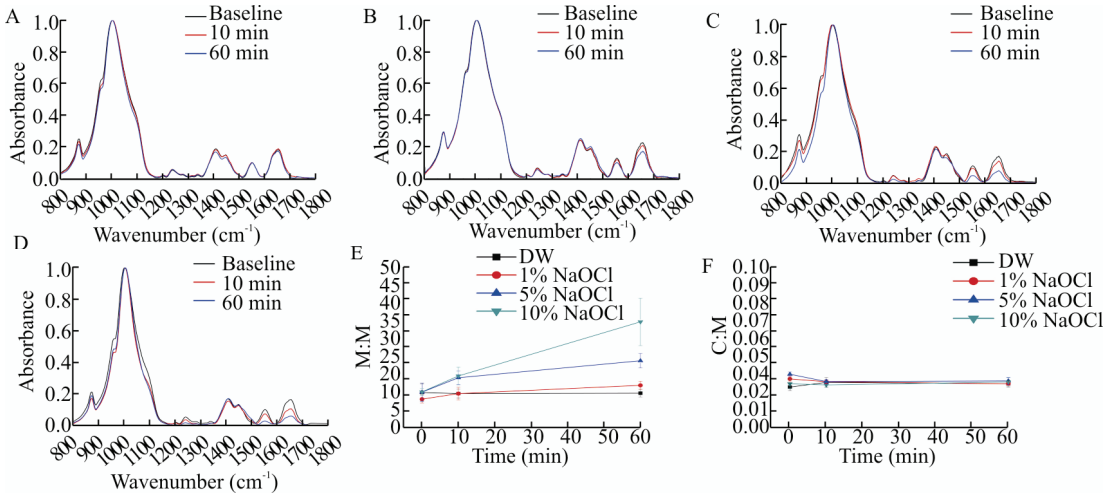

Fig. 2 ATR-IR spectra of dentin before and after treatment

A: DW group; B: 1% NaOCl group; C: 5% NaOCl group; D: 10% NaOCl group. E, F: changes of the M:M (E) and the C:M (F). The M:M remained unchanged in DW group ($P>0.05$), but it increased with time in NaOCl groups ($P<0.05$). The C:M was unchanged in all groups ($P>0.05$).

Further analysis revealed that the M:M remained unchanged after treatment in the DW group, but it increased with time in the NaOCl groups (fig. 2E). Two-way RMANOVA showed statistically significant differences in the M:M between the NaOCl groups and control group. In 1% NaOCl group, significant differences in the M:M were found between 60 min and baseline or 10 min (P<0.05), but there was no difference between baseline and 10 min (P>0.05). In 5% and 10% NaOCl groups, significant differences were noted in the M:M between any two time points (P<0.05). Additionally, at 10 min, 10% NaOCl group had significantly increased M:M when compared with DW group or 1% NaOCl group (both P<0.05), while the M:M in 10% NaOCl group was not significantly different from that in 5% NaOCl group (P>0.05). No significant difference in the M:M was found among DW group, 1% NaOCl group and 5% NaOCl group at 10 min (P>0.05). At 60 min, the M:M was much greater in 10% NaOCl group than in DW group, 1% NaOCl or 5% NaOCl group (P<0.01, P<0.01 and P<0.05, respectively). There was no significant difference in the M:M among DW group, 1% NaOCl group and 5% NaOCl group at this time point (P>0.05).

The C:M remained practically unchanged after treatment in all groups (fig. 2F). Two-way RMANOVA showed the treatment time and the treatment concentrations had no significant effects on the C:M (P>0.05 and P>0.05, respectively).

2.2 Raman Spectrum Analysis

The characteristic Raman spectra of dentin before and after treatment are displayed in fig. 3A–3D. The strongest band at 960 cm^{-1} was attributed to the symmetric stretching mode of $v_1PO_4^{-3}$, at 1045 cm^{-1} and 1024 cm^{-1} to $v_3PO_4^{-3}$, at 610 cm^{-1} and 580 cm^{-1} to $v_4PO_4^{-3}$, and at 430 cm^{-1} to $v_2PO_4^{-3}$. The peaks at 1068 cm^{-1} and 3573 cm^{-1} were attributed to $v_2CO_3^{-2}$ and OH^- stretch, respectively. The laser-induced fluorescence of dentin appeared as a featureless background in the Raman spectra.

The frequency and bandwidth of $v_1PO_4^{-3}$ showed little variation after treatment in all groups (fig. 3A–3D). Further analysis revealed that the percentage of RRI remained unchanged (fig. 3E). Two-way RMANOVA showed that the treatment time or the treatment concentration factor had no significant effects on the percentage of RRI (P>0.05 and P>0.05, respectively).

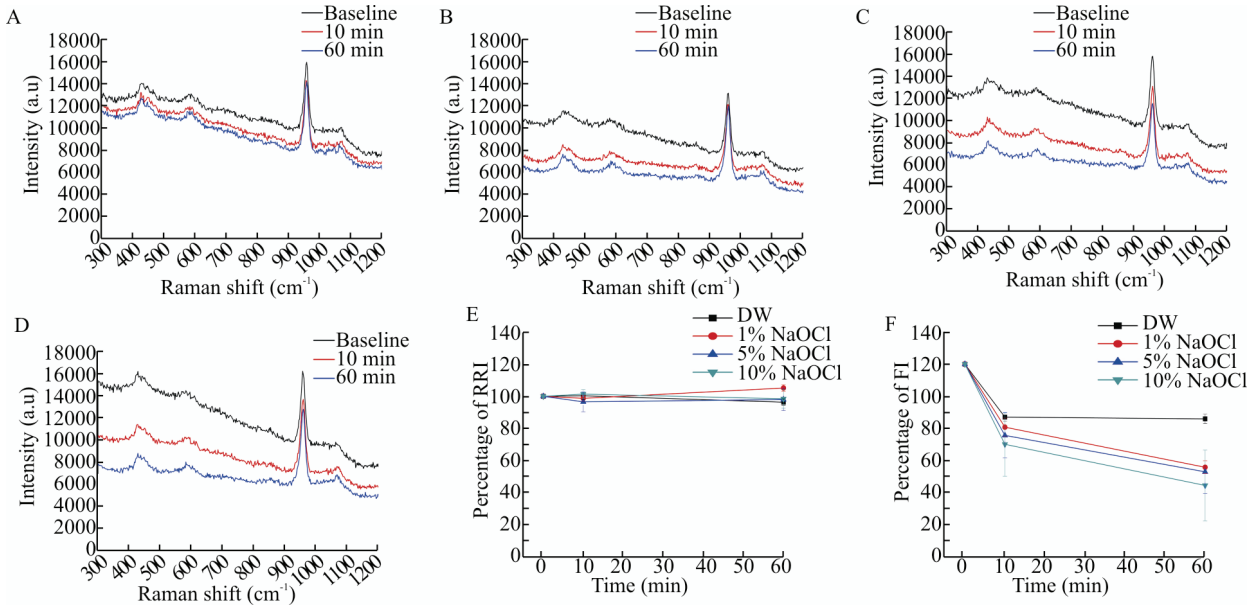

Fig. 3 Raman spectra of dentin before and after treatment

A: DW group; B: 1% NaOCl group; C: 5% NaOCl group; D: 10% NaOCl group. E, F: Changes of percentage of RRI and FI. The percentage of RRI remained unchanged in all groups (P>0.05). The percentage of FI declined with time in all groups (P<0.05).

As shown in fig. 3F, the percentage of FI tended to decline with time in all groups. Two-way RMANOVA showed the treatment concentrations had no significant effect on the percentage of FI (P>0.05), whereas statistically significant differences were observed in the percentage of FI between different time groups (P<0.01). It was noticed that the percentage of FI in DW group decreased at 10 min as compared with baseline (P<0.01), but it remained almost unchanged between 10 min and 60 min (P>0.05). However, for the NaOCl groups, the percentage of FI decreased significantly from baseline to 10 min (P<0.01, P<0.05 and P<0.05 respectively), and continually from 10 min to 60 min (P<0.05, P<0.01 and P<0.01 respectively).

2.3 XRD Spectra Analysis

Fig. 4 displays the typical XRD patterns for dentin. FWHM, a-axis and c-axis length of the crystal apatite didn't change profoundly after treatment in all groups (table 1–3), and there was no significant difference in XRD between different treatment time groups and between different treatment concentration groups (both P<0.05).

Table 1 Mean values (standard deviations) of the FWHM from XRD in each group

Groups	Baseline	10 min	60 min
DW	0.411 (0.020)	0.410 (0.037)	0.416 (0.013)
1% NaOCl	0.440 (0.094)	0.418 (0.039)	0.387 (0.015)
5% NaOCl	0.358 (0.041)	0.390 (0.021)	0.337 (0.028)
10% NaOCl	0.407 (0.059)	0.386 (0.032)	0.428 (0.014)

Table 2 Mean values (standard deviations) of the a-axis of apatite from XRD in each group

Group	Baseline	10 min	60 min
DW	9.395 (0.018)	9.384 (0.010)	9.401 (0.006)
1% NaOCl	9.385 (0.013)	9.391 (0.014)	9.389 (0.004)
5% NaOCl	9.392 (0.016)	9.398 (0.027)	9.386 (0.008)
10% NaOCl	9.404 (0.008)	9.393 (0.007)	9.387 (0.017)

Table 3 Mean values (standard deviations) of the c-axis of apatite from XRD in each group

Group	Baseline	10 min	60 min
DW	6.887 (0.008)	6.878 (0.015)	6.901 (0.026)
1% NaOCl	6.886 (0.021)	6.881 (0.013)	6.882 (0.004)
5% NaOCl	6.877 (0.025)	6.879 (0.018)	6.886 (0.015)
10% NaOCl	6.866 (0.005)	6.892 (0.012)	6.891 (0.008)

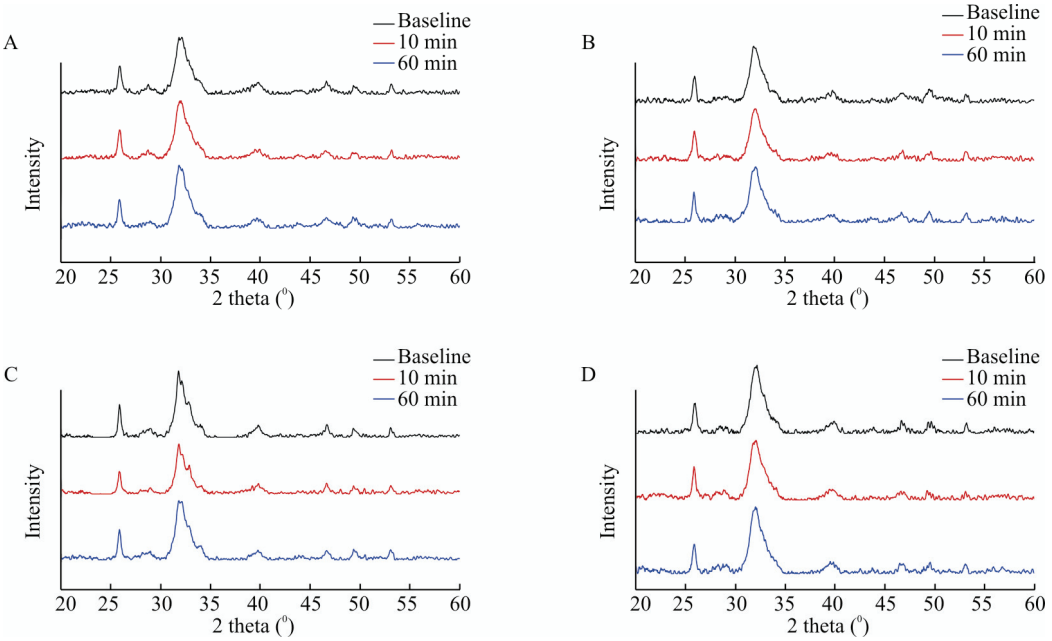

Fig. 4 XRD spectra of dentin before and after treatment
A: DW group; B: 1% NaOCl group; C: 5% NaOCl group; D: 10% NaOCl group

2.4 SEM Images Analysis

After 10 min, the specimens displayed intact dentinal tubules, normal networks of tubular walls and smooth, flat intertubular dentin surface in the DW group (fig. 5A). However, in the NaOCl groups, they revealed irregular dentinal tubules, more crystal granules, less networks of tubular walls and rough intertubular dentin surface, regardless of various concentrations of NaOCl (fig. 5B–5D). In terms of the intertubular dentin, the specimens showed shallow and subtle erosion-like traces in 1% NaOCl group (fig. 5B3), whereas in 5% or 10% NaOCl group, deeper traces were observed (fig. 5C3–5D3).

After 60 min treatment, the specimens still displayed little variation in the DW group (fig. 6A). However, in the NaOCl groups, they revealed a loss of integrity of the dentinal structure and an emergence of tiny channels around dentinal tubules (fig. 6B–6D). Especially in 10% NaOCl group, there was an apparent exposure of the subsurface dentinal structures and a presence of relatively distinguishable crescent-shaped periturbular dentin (fig. 6D1). Besides, the networks of tubular walls disappeared and numerous crystal granules were left, regardless of various concentrations of NaOCl (fig. 6B2–6D2). In terms of the intertubular dentin, obvious erosion-like traces appeared on the surface of the

specimens in all the NaOCl groups (fig. 6B3–6D3).

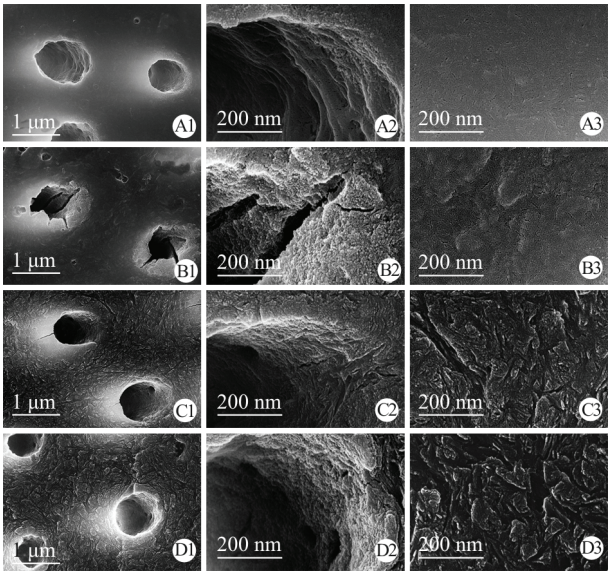

Fig. 5 SEM images of dentin surface at 10 min
A: DW group; B: 1% NaOCl group; C: 5% NaOCl group; D: 10% NaOCl group at magnifications of 10 000× (-1) and 50 000× (-2, -3). The specimens displayed intact dentinal tubules, normal networks of tubular walls and smooth, flat intertubular dentin surface in DW group, while they revealed irregular dentinal tubules, more crystal granules, less networks of tubular walls and rough intertubular dentin surface in NaOCl groups.

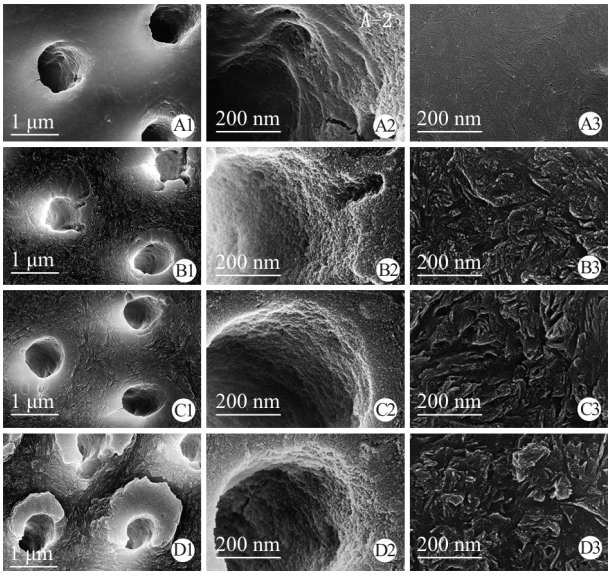

Fig. 6 SEM images of dentin surface at 60 min
A: DW group; B: 1% NaOCl group; C: 5% NaOCl group; D: 10% NaOCl group at magnifications of 10 000× (-1) and 50 000× (-2, -3). The specimens displayed little variation in DW group, while they revealed a loss of networks of tubular walls, an emergence of tiny channels around the dentinal tubules, numerous crystal granules along the walls and obvious erosion-like traces on the intertubular dentin surface in NaOCl groups.

2.5 Flexural Strength Analysis

The changes of flexural strength of dentin bars are exhibited in fig. 7. After treatment, the flexural strength decreased in all the NaOCl groups rather than in the DW group. Two-way RMANOVA showed statistically significant differences in the flexural strength between different treatment time groups and between different treatment concentration groups (both $P<0.01$). The flexural strength remained unchanged between any two time points in DW or 1% NaOCl group ($P>0.05$). In 5% and 10% NaOCl groups, it dropped dramatically from baseline to 10 min ($P<0.05$), and then kept stable from 10 min to 60 min ($P>0.05$). For the different treatment concentrations, at 10 min, significant differences were noted in the flexural strength among all groups ($P<0.05$). At 60 min, no significant difference was detected between 1% and 5% NaOCl groups ($P>0.05$), but there were significant differences between other groups ($P<0.05$).

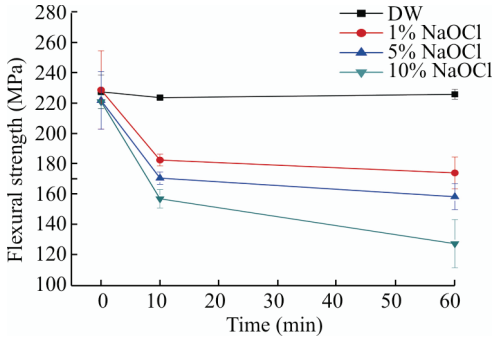

Fig. 7 Flexural strength in each group
Flexural strength remained unchanged in DW and 1% NaOCl groups ($P>0.05$), but it decreased with time in 5% and 10% NaOCl groups ($P<0.05$).

3 DISCUSSION

Non-invasive detection techniques help us obtain the real-time data with less bias and analyze the chemical structure of biological specimens at the molecular level. In the present study, we evaluated the effects of different concentrations and exposure time of NaOCl on the structure, compositional and mechanical properties of human dentin mainly by means of ATR-IR and Raman spectroscopy *in vitro*.

Raman spectroscopy, with its noninvasive and powerful advantages, easily obtained the valuable information regarding the mineral quantity of dentin. In this study, it was shown that the RRI value did not change significantly at different time points in each group. Since RRI derived from the Raman spectra is linearly proportional to the phosphate group concentration within hydroxyapatite molecules[19] and phosphate group concentration is a good indicator of the mineral content in dentin[22, 23], the unchanged RRI value indicated that the mineral of dentin was less affected by NaOCl treatment. Our finding demonstrated that the treatment of 1% or 10% NaOCl within 60 min barely changed the mineral content in dentin.

Besides, the dentinal mineral crystals were analyzed by means of ATR-IR and XRD. The C:M from ATR-IR spectra showed little alteration in each group. The presence of carbonate is an important modification in the hydroxyapatite lattice[17, 24], which can reduce the crystallinity of apatite, limit the size of crystals and then influence their shape by distorting the apatite lattice[25–28]. The unchanged C:M still indicated that the mineral was

stable in NaOCl-treated dentin. XRD also yielded the crystallographic information about human dentin. The FWHM, a-axis and c-axis were found to be nearly the same as the baseline level, even after the treatment with high concentration of NaOCl (10%). As these parameters reflect the crystallinity and crystal size of dentinal mineral crystals[28, 29], it was demonstrated that NaOCl caused little effects on the crystallization of human dentin. However, Sakae *et al* had suggested that NaOCl was capable of removing magnesium and carbonate ions from dentin[30]. Borges *et al* found that 1% NaOCl was sufficient to change the molecular arrangement of inorganic dentin content[31]. The discrepancy might be attributed to the diverse states of specimens for XRD detection and the different sensitivities of test parameters.

It is well known that dentin consists of both mineral (70% wt.) and organic (20% wt.) content. We found that the dentinal organic content changed after NaOCl treatment. The M:M from ATR-IR spectrum could be reasonably used to analyze the alteration of dentinal organic content. It was found that the M:M was relatively stable in the DW group, but it increased with time in the NaOCl groups. Amide peaks stand for the protein conformation in dentin[32]. The rise of the M:M provided direct spectroscopic evidence that the organic content was undermined and eliminated time-dependently by NaOCl. Additionally, the M:M increased with the concentration, which demonstrated a concentration-dependent effect of NaOCl on dentinal organic content.

SEM observations illustrated the destruction of the dentinal structure by NaOCl from a morphological perspective. Unlike the smooth, flat and polished surface of specimens treated only with DW, varying degrees of erosion-like traces appeared on the surface in NaOCl groups. More tiny channels around the dentinal tubules after NaOCl treatment at the magnification of 10 000× demonstrated that NaOCl had penetrated the dentinal structure and made the underlying layer exposed. Furthermore, the loss of networks and the presence of abundant granules along the tubular walls after NaOCl treatment at the magnification of 50 000× revealed that the meshed collagen was degraded and the deproteinized hydroxyapatite was left in the cross-section pattern. Comparing the images at different time points of the same group, it was clear that erosion-like traces at 60 min was deeper and wider than those at 10 min. This indicated that NaOCl posed a time-dependent effect on the dentinal organic content. Besides, the concentration-dependent effect was also obvious as evidenced by the fact that the erosion-like traces on the intertubular dentin in 5% and 10% NaOCl groups was deeper and wider than those in 1% NaOCl group at the same time point.

In fact, the removal of the exposed collagen matrix by NaOCl was reported by previous studies[10, 12, 13]. It is known that NaOCl is a strong base. Its pH value decreases by releasing hydroxyl ions, forming water and salt when exposed to amino acids. Also, hypochlorous acid from NaOCl acts as a solvent, when it meets the organic content. Hydroxyl ions and hypochlorous acid lead to the degradation and hydrolysis of amino acid.

However, NaOCl of a low concentration within a short time was insufficient to cause the structural variations on dentin. It was noted that in 1% NaOCl group, the M:M from ATR-IR spectra remained nearly unchanged from baseline to 10 min, which was in agreement with of the results of SEM, in which the erosion-like traces in 1% NaOCl group at 10 min was relatively unobvious. These findings demonstrated that treatment of 1% NaOCl within 10 min could not result in structural changes, either of the inorganic mineral or the organic content, in human dentin. Therefore, an irrigation of 1% NaOCl within 10 min is probably suitable to minimize its effects on human dentin during root canal treatment from the structural perspective.

Another important finding in the present study is the FI alterations from Raman spectra. A weak but significant FI reduction was observed in DW group in the first 10 min, and then FI remained nearly unchanged to 60 min. According to a previous study[19], such drop was probably due to the effect of "photo bleaching", as the specimens were repeatedly measured at the same site. In NaOCl groups, FI remarkably decreased not only in the first 10 min, but also at 60 min. These phenomena indicated that except for the "photo bleaching" effect, a specific matter in human dentin, which could emit fluorescence, declined after the treatment of NaOCl. As mentioned above, dentinal mineral crystals barely changed quantitatively and qualitatively in NaOCl groups, this matter cannot be the crystal. Therefore, FI might provide information of a bulk organic matter in dentin, like collagens; or a trace of organic matter between collagens and mineral crystals in dentin, like non-collagenous proteins (NCPs). This speculation is supported by previous studies examining the dentinal fluorescence[33, 34], which suggested that the laser-induced fluorescence originated from the organic matter in dentin. However, it is still difficult to trace the exact origin of the fluorescent material in human dentin according to the present results of our study. Further investigations are warranted to examine whether particular kinds of collagens or NCPs or their combination contribute to the FI alterations.

We found that NaOCl made a concentration- and time-dependent reduction on flexural strength of dentin through three-point loading tests. This result was consistent with the ATR-IR result. The treatment of 1% NaOCl for 10 min did not lead to any significant rise in the M:M or any obvious drop in flexural strength, whereas treatment with a longer time or a higher concentration remarkably increased the M:M and decreased the flexural strength. Based on the similar results from the two different detection methods, we demonstrated that NaOCl destroyed the organic matrix of dentin, and hence weakened its flexural strength. As it is known that flexural strength reflects the toughness[3, 35], our study also suggested that the organic matrix of dentin played a profound role in its toughness. The only difference between the results of ATR-IR and flexural strength was, the M:M in 1% NaOCl group at 60 min was significantly greater than that at baseline, while the flexural strength in the same group at the same time was not. This may be attributed to the various sensitivity thresholds of the diverse techniques. In other words, the structural alterations for this level was not large enough to cause obvious changes in the flexural strength.

To sum up, irrespective of the limitations of this *in vitro* study, it was suggested that NaOCl has no signifi-

cant effects on the inorganic mineral of human dentin; however, it undermines and eliminates the organic content concentration- and time-dependently, which in turn influences its flexural strength and toughness. In addition, an irrigation of 1% NaOCl within 10 min could minimize the effects of NaOCl upon the structural and mechanical properties of dentin during root canal treatment.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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