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Original Article

In vitro evaluation of the impact of a bioceramic root canal sealer on the mechanical properties of tooth roots

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Abstract *Bacground/purpose:* Endodontically treated teeth are more prone to vertical root fracture with the mechanical property changes to some extent during root canal treatment. This study aimed to investigate the effects of a bioceramic sealer on the mechanical properties of tooth roots.
Materials and methods: Dentin discs were dried by two different methods (ethanol drying and paper points drying) and then filled with a BC sealer named iRoot SP. SEM and EDS were used to analyze the newly formed minerals in dentin tubules. Elastic modulus and hardness of the secondary dentin in areas proximal to the primary dentin (PD-SD) and areas proximal to canal or iRoot SP (SD-C/SD-iRoot SP) were measured using nanoindentation technique. The compressive strength of roots filled with iRoot SP were tested by compressive loading test.
Results: (1) Penetration and mineralization: Paper points drying was more conducive to iRoot SP adhesion, spreading and penetration into the dentin tubules than 95% ethanol drying. (2) Micromechanical properties: After filling root canal with iRoot SP, the elastic modulus and hardness of SD-iRoot SP were higher than those of PD-SD ($P = 0.001$ and $P = 0.000$). (3) Fracture resistance: The compressive strength of the roots filled with iRoot SP was not significantly different from that of the roots unprepared and unfilled ($P = 0.957$), but was higher than that of the roots prepared and unfilled ($P = 0.009$).
Conclusion: Excessive drying (95% ethanol drying method) is not conducive to the penetration and mineralization of the BC sealer iRoot SP into dentin tubules. The good bioactivity of iRoot SP was responsible for increasing the elastic modulus and hardness of dentin, which strengthened the prepared roots.

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Introduction

Vertical root fracture is a chronic injury disease of teeth with a prevalence of 2%—32%,[1—3] which is more common in teeth that have undergone root canal treatment.[2,4,5] Studies have shown that while removing infection by mechanical and chemical preparation, root canal therapy can also weaken the resistance of the tooth's root, resulting in the decreased flexural strength, elastic modulus and microhardness.[6—9] When it occurs, endodontically treated teeth may have to be extracted.

In recent years, bioceramic (BC) sealers with calcium silicate as the main component used in clinical practice exhibit no shrinkage during curing, and even demonstrate a slight volume expansion.[10] Additionally, they possess good dentin tubule permeability,[11,12] which enables effective micromechanical interlocking with root dentin. At the same time, they are hydrophilic and exhibit good bioactivity. When contacting with a liquid containing phosphate ions, they can undergo hydration reaction to first form calcium hydroxide, and then apatite on their surface, which can combine with root dentin.[13] Several studies have demonstrated that root canal filling with BC sealers enhanced the fracture resistance of the prepared root,[14—16] although the mechanism remains unclear.

In dentin, the strength of the root is determined by inorganic hydroxyapatite, while the toughness of dentin is primarily determined by type I collagen, which constitutes the majority of the organic matrix.[17,18] Pires et al. pointed out that MTA with calcium silicate as the main component can induce apatite deposition in demineralized dentin and restore its hardness.[19] Therefore, we hypothesized that bioceramic sealers could mineralize after penetrating into the dentin tubules and altered the micromechanical properties of dentin, thereby enhancing the fracture resistance of the root after endodontic treatment. iRoot SP (Innovative Bioceramix, Vancouver, Canada), also called Endosequence BC Sealer (Brasseler, Savannah, GA, USA), is a premixed, ready-to-use, and injectable sealer. It is a representative BC sealer with good physical and chemical properties, also biological properties mentioned above.[10—16] This study aimed to investigate the mineralization effect of the bioceramic sealer iRoot SP in root canals and its effects on the mechanical properties of root dentin.

Materials and methods

The study was approved by the Biomedical Institutional Review Board of Peking University School and Hospital of Stomatology (PKUSSIRB-201631102). A total of 40 adult teeth with single root and single canal extracted due to periodontitis or orthodontic reasons were included in this study. All the extracted teeth were examined under a 10 × field of view stereomicroscope, and the teeth with immature root, root surface caries, root fracture, and root resorption were excluded. The periodontal ligament and calculus deposits on the root surface were scraped with a scaler and stored in normal saline in a 4 °C refrigerator.

Assessment of mineralization performance

The crowns of eight teeth with single root and single root canal were removed at the cementoenamel junction. Each specimen was embedded using cold self-curing resin and then sectioned perpendicular to its long axis using a 0.3 mm low-speed saw (Shenyang Kejing Autoinstrument, Shenyang, China) at 200 rpm with continuous water cooling. Two dentin discs with thickness of 2 mm were obtained from the middle third of every root and then stored in normal saline solution.

Sixteen dentin discs without obvious defects and cracks were selected for the experiment. The root canals were prepared with a 4#GG drill (d = 1.1 mm) and rinsed with running water, then immersed in 2.5% NaClO and 17% EDTA for 3min, respectively. All the dentin discs were divided into two groups: one was *paper points drying group*, in which the root canals were dried with paper points until the last paper point was dry. The other group was *ethanol drying group*, in which the root canals were first dried with paper points, then the dentin discs were immersed in 95% ethanol for 10s, and then paper points dried again. Each group was then randomly divided into four subgroups, with two dentin discs in each subgroup, respectively:

Control group: Root canals were only prepared without root canal filling. Then the samples were stored in 37 °C 100% relative humidity for 1 day; *Control-SBF group*: the root canals were prepared without root canal filling and immersed in SBF for 4 weeks, and the solution was changed every two days.

iRoot SP group: The root canals were filled with the bioceramic material iRoot SP after root canal preparation and solidified at 37 °C and 100% relative humidity for 1 day.

iRoot SP-SBF group: The root canals were filled with iRoot SP after root canal preparation, solidified at 37 °C and 100% relative humidity for 1 day, immersed in SBF for 4 weeks, and the solution was changed every two days.

The dentin discs were frozen in liquid nitrogen for 24 h and then immediately broken into two parts along the buccal-lingual groove prepared in advance. The discs were desiccated at room temperature and sprayed with gold. The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) (Hitachi S-4800, Tokyo, Japan) were used to observe the morphology and elemental composition of newly formed minerals in dentin tubules.

Measurement of dentin elastic modulus and hardness

Two 4-mm dentin discs were obtained from the middle third of two roots according to the above method, which were named sample A and sample B, respectively. Sample A was subjected to mechanical canal preparation without filling. That is, root canals were firstly prepared with Protaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) after gradual preparation to F3, and then a 4#GG drill was used to prepare it to a diameter of 1.1 mm and rinsed with running water. Sample B was mechanically prepared to 1.1 mm in diameter, then immersed in 2.5%NaClO for 3min and 17%EDTA for 3min to remove the smear layer. After washing with distilled water, the root canal was dried with paper points. iRoot SP was filled into the root canal and then solidified at 37 °C and 100% relative humidity for 1 day, immersed in SBF for 4 weeks. The solution was changed every two days.

Small amounts of minerals deposited on the surface of sample B were removed by polishing with 2000 and 4000 mesh silicon carbide abrasive paper. After polishing both sample A and sample B with a metallographic sample polishing machine under water cooling, the surface structure was observed under a metallographic microscope to ensure that the dentin structure could be clearly observed under a 1000 × field of view and the dentin surface was smooth without polishing mark. Then, the samples were placed in D 'Hanks balanced salt solution for further use.

The secondary dentin at 20–30 μm proximal to the root canal or iRoot SP (secondary dentin-canal/secondary dentin-iRoot SP, SD-C/SD-iRoot SP) (Fig. 1, black box) and the secondary dentin at 45 μm proximal to the primary dentin side (primary dentin-secondary dentin, PD-SD) (Fig. 1, red box) were selected under the light microscopy of the in situ nanomechanical testing system (TI-900 TriboIndenter, Hysitron, Minneapolis, MN, USA) and were used as the measurement areas to test the elastic modulus and hardness of the intertubular dentin in the corresponding areas.

The samples were placed on the stage and tested on the polished dentin surface with a Berkovich diamond indenter with a 50 nm tip radius. The scanning probe microscopy (SPM) of the in-situ nanomechanical testing system was used to scan and image the intertubular dentin. Then cyclic loading was carried out with loads ranging from 0 to 10 mN, and the first load when stable elastic modulus and hardness were obtained was set as the load of subsequent quasi-static loading, which was 2000 μN. The test was performed with a load of 2000 μN in the target area, maintained for 2s between loading and unloading, and every two loading sites were not less than 2 times the indentation size apart. After a selection region test was completed, an in situ imaging scan with SPM was performed to confirm the indentation location, and imaging was recorded. After the test, the load-displacement curve, shape and position of the indentation were observed. The data with correct position, complete shape and normal load-displacement curve of the indentation were selected for statistical analysis, and the data with indentation into the dentin tubules or incomplete shape were discarded to ensure the authenticity and reliability of the results. Finally, sample A and sample B obtained valid data for 11 sets of SD-C/SD-iRoot SP and PD-SD regions, respectively.

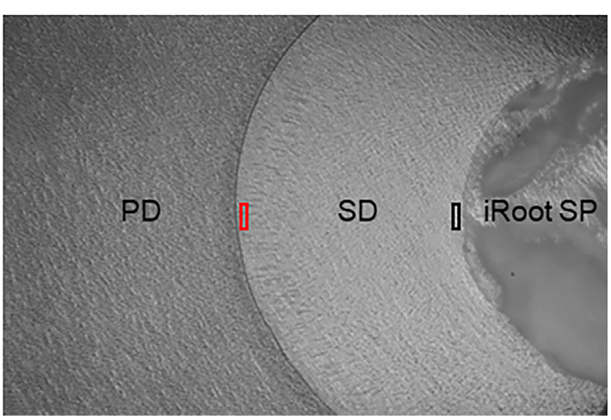

Figure 1 Measurement areas for nanoindentation test. PD, primary dentin; SD, secondary dentin; iRoot SP, the bioceramic sealer filled in the root canal. The red box refers to the secondary dentin adjacent to the primary dentin (PD-SD) and the black box refers to the secondary dentin proximal to the empty root canal or iRoot SP (SD-C/SD-iRoot SP).

Measurement of the compressive strength of the roots

Thirty mandibular premolars with single root and single canal, whose initial apical file was no larger than SS15#K file, were selected. The crowns were removed at the apical direction of cementoenamel junction using a 0.3 mm low-speed diamond saw and obtained roots with a standardized length of 13 mm. The working length was determined by subtracting 1 mm from the length of a size 15 K-file until it reached the apical foramen. Then the roots were randomly divided into the following three groups (n = 10 each) named as blank group (unprepared and unfilled), unfilled group (prepared and unfilled), and iRoot SP group (prepared and filled with iRoot SP), respectively. In unfilled group and iRoot SP group, each root canal was instrumented using rotary Ni–Ti instruments ProTaper Universal at the working length until the F3 instrument. After each instrument was used, the canals were irrigated using 2 mL 2.5% sodium hypochlorite. Then, passive ultrasonic irrigation with 2.5% sodium hypochlorite was performed. A flush of 2 mL 17% EDTA was applied for 3 min to eliminate the smear layer. Finally, the canals were rinsed with 2 mL distilled water and dried with paper points. In iRoot SP group, the root canals were injected with iRoot SP until 1 mm below the root canal orifice, and then filled with a temporary filling material Ceivitron (Dongquan, Taibei, China) and taken periapical radiographs to ensure that the canals were densely filled and free of bubbles.

All samples were placed at 37 °C, 100% relative humidity for 1 week to allow the sealer to set, and the samples were immersed in SBF for another 4 weeks with solution changes every two days.

A 2 mm wide tape was used to wrap around the coronal edge of the sample as the embedding boundary, and the long axis of the root was perpendicular to the ground and the underlying 11 mm was embedded with cold self-curing resin. A hemispherical stainless steel compression head with a diameter of 3 mm was fixed to the clamp of a universal testing machine (Instron, Boston, MA, USA), which was pressurized perpendicular to the root section at a rate of 1 mm/min. The maximum load force (N) was recorded as the compressive load when the root was suddenly fractured. The compressive strength is calculated according to the following formula: $C = 4F/\pi d2$, where C is the compressive strength (MPa), F is the maximum load force (N), d is the radius of sealers (mm), and $\pi = 3.14$.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation and analyzed by SPSS 16.0 statistical software. The data of elastic modulus and hardness of dentin were analyzed by paired sample *t*-test. The compressive load and compressive strength of roots were analyzed by homogeneity test of variance and one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Effects of different drying methods on the penetration and mineralization of iRoot SP in dentin

After drying the canals with different methods and filling them with iRoot SP for 1 day, SEM observation showed that paper points drying method was more conducive to iRoot SP adhesion, spreading and penetration into the dentin tubules than 95% ethanol drying. Although the smear layer was removed and the dentin tubules were open after the root canal was dried with 95% ethanol (Fig. 2A–C), iRoot SP attached little to the surface of the root canal wall and failed to completely cover the surface of the root canal (Fig. 2D–F), with only a few spherical granules of iRoot SP scattering in the dentin tubules. Whereas in root canal dried with paper points, iRoot SP adhered and spread well on the surface of the root canal wall, covering almost all openings of the dentin tubules (Fig. 2J-L). A large number of iRoot SP entered into the dentin tubules and gathered and condensed with each other to form a complex structure. However, the structure was relatively loose, and spherical particle structure was still visible on the surface (Fig. 2 K, L).

After the root canals were dried by different methods and immersed in SBF for 4 weeks, even without filling, SEM observation showed that the surface of the dentin tubules was covered by a thin layer of meshed new minerals with uniform structure, regardless of whether the root canals were dried with 95% ethanol or with paper points (Fig. 2 M – O, S–U). However, the mineral deposition in the dentin tubules increased significantly after iRoot SP filling (Fig. 2 P–R, V-X). In the iRoot SP-filled samples, the minerals in the 95% ethanol drying group did not full fill the dentin tubules and showed clear spherical granular structures, which were stacked closely together but did not form a whole structure (Fig. 2P–R). In the paper points drying group, the process formed by iRoot SP entering the dentin tubule was observed, which completely duplicated the shape of the dentin tubule (Fig. 2W). At the same time, new minerals were formed on the surface of iRoot SP, and spherical particles were visible on the surface which grew along the dentin tubules and covered the structure of the dentin tubules (Fig. 2V–X). By EDS analysis, the new minerals in the paper points drying group were composed of Ca, P, O, Si, and C elements, and the Ca/P ratio was 1.95, while the P element was not detected in the minerals in the 95% ethanol drying group. These results indicated that drying root canals with 95% ethanol was not conducive to the mineralization of iRoot SP in dentin tubules. However, when the root canals were dried with paper points, iRoot SP showed a higher degree of mineralization within dentin tubules.

The effect of iRoot SP on the elastic modulus and hardness of root canal dentin

Table 1 showed the dentin elastic modulus and hardness in different measurement areas of samples A and B. For sample A without root canal filling, there was no significant difference in elastic modulus and hardness between PD-SD and SD-C ($P = 0.093$ and 0.666, respectively). However, in sample B with iRoot SP filling, the elastic modulus and hardness of the intertubular dentin at the region of SD-iRoot SP were both higher than those of the intertubular dentin at region of PD-SD ($P = 0.001$ and 0.000, respectively), indicating that filling root canal with iRoot SP increased the elastic modulus and hardness of intertubular dentin.

The effect of iRoot SP on the compressive strength of the roots

As shown in Table 2, the compressive load and strength of the roots filled with iRoot SP were not significantly different from those of the blank group ($P = 0.957$), but were significantly higher than those of the unfilled group ($P = 0.009$). The results showed that filling root canal with iRoot SP improved the fracture resistance of prepared roots.

Discussion

The results of this study showed that the bioceramic sealer iRoot SP could enter the dentin tubules, form a mineralization reaction and improve the elastic modulus and hardness of the dentin, thereby improving the fracture resistance of the root after root canal treatment.

In this study, 17%EDTA was used to treat dentin, which caused incomplete demineralization of dentin and partial exposure of collagen fibers while the smear layer was removed to open the dentin tubules. The organic matter in dentin is 90% collagen and 10% non-collagen. Most non-collagenous proteins are phosphoproteins, which are negatively charged and may be associated with biomineralization. Kawasaki et al. reported that phosphorylated proteins in the dentin matrix were able to regulate crystal nucleation and growth, as well as binding to the collagen network. It has been hypothesized that the insoluble phosphoproteins in decalcified collagen could serve as sites for mineral nucleation and undergo remineralization.[20] In this study, the control group without root canal filling also

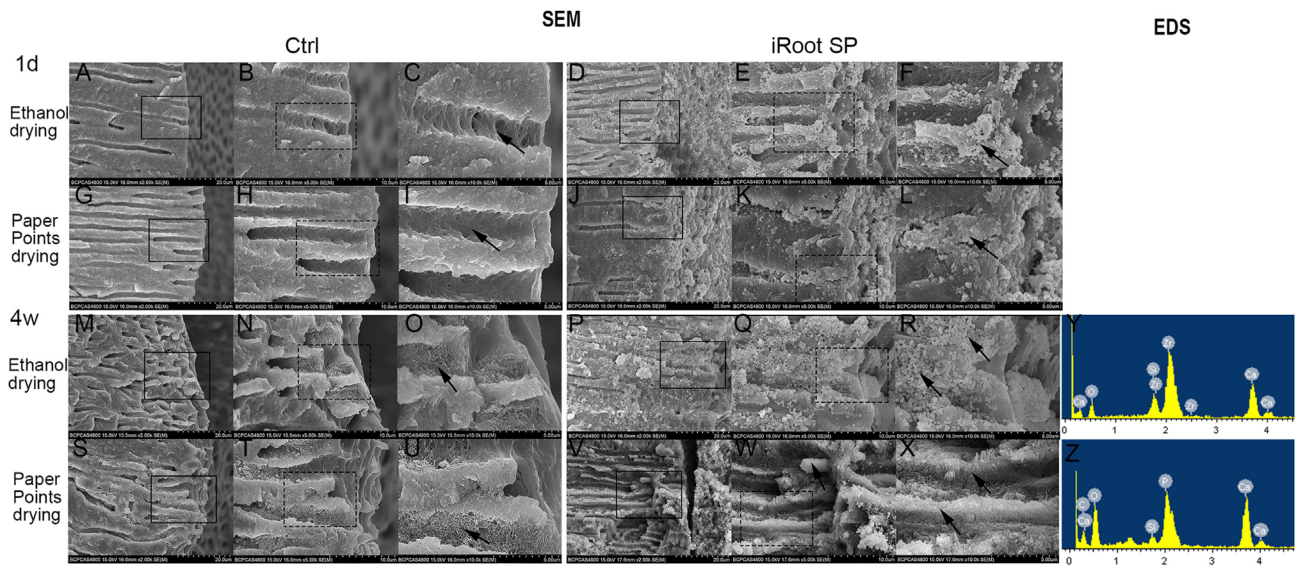

Figure 2 Effect of different drying methods on the penetration and mineralization properties of iRoot SP in dentin by SEM. The right column (10k $\times$) was the amplification of the dotted box in the middle column (5k $\times$). The middle column (5k $\times$) was the amplification of the dotted box in the left column (2k $\times$). The arrows in (C) and (I) showed exposed and collapsed collagen fibers; Arrows in (O) and (U) indicated thin layers of minerals deposited in dentin tubules; The arrow in (F) showed a small amount of iRoot SP penetrating into the dentin tubules, showing scattered spherical granules without forming a complex structure. The arrow in (L) showed that a large number of iRoot SP penetrate into the dentin tubules, and the spherical particle structure can be seen. The arrow in (R) shows the spherical particles formed by a small amount of iRoot SP mineralization. The arrow in (W) showed the process formed by iRoot SP penetrating into the dentin tubule, which completely replicates the shape of the dentin tubule. The arrows in (X) indicate that iRoot SP in dentin tubules are mineralized and the surface is covered with new minerals. (Y) and (Z) are EDS profiles of the filling material in (R) and the nascent minerals in (X), respectively.

Table 1 The elastic modulus and hardness of intertubular dentin measured by nanoindentation technique (n = 11).

Group	Site	Elastic modulus (GPa)	*P*-value	Hardness (MPa)	*P*-value
Sample A	PD-SD	21.96 ± 2.31	0.093	773.22 ± 50.62	0.666
	SD-C	20.80 ± 2.01		794.89 ± 153.59	
Sample B	PD-SD	16.50 ± 1.61	0.001	378.18 ± 39.68	0.000
	SD-iRoot SP	19.65 ± 1.66		647.27 ± 74.89	

Abbreviations: PD-SD, primary dentin-secondary dentin, refers to the secondary dentin adjacent to the primary dentin; SD-C, secondary dentin-canal, refers to the secondary dentin adjacent to the root canal without iRoot SP; SD-iRoot SP, secondary dentin-iRoot SP, refers to the secondary dentin proximal to iRoot SP.

had a small amount of mineral formation on the dentin tubule wall after soaking in SBF by SEM. It may be because under the action of non-collagen, calcium and phosphorus ions entered the incompletely demineralized dentin matrix and deposited on the surface forming a thin mineral layer. This was similar to the results of Toledano,[21] who pointed out that demineralized dentin is a bioactive substrate, and hydroxyapatite could be deposited on its surface as early as about 30 min.

Table 2 Compressive load (N) and compressive strength (MPa) of the roots in different groups (n = 10).

Group	Compressive load (N)	Compressive strength (MPa)
Blank group	945.36 ± 180.99[a]	133.74 ± 25.60[a]
Unfilled group	748.37 ± 127.03[b]	105.87 ± 17.97[b]
iRoot SP group	949.30 ± 156.78[a]	134.30 ± 22.18[a]

Abbreviations: Blank group, unprepared and unfilled; Unfilled group, prepared and unfilled; iRoot SP group, prepared and filled with iRoot SP.
Different letters represented significant differences between groups ($P < 0.05$).

The minerals in the dentin tubules were significantly increased in iRoot SP group compared with the control group. The mineral had iRoot SP at one end and dentin matrix at the other end, indicating that iRoot SP had good biological activity. This is inseparable from its chemical composition and the role of the surrounding environment. As a BC sealer, iRoot SP entered dentin tubules and underwent hydration reaction. After Ca^{2+}-H^{+} ion exchange, SiO^{-} formation, interaction between SiO^{-} and Ca^{2+} charge, formation of mineralized induced surface, and deposition of amorphous calcium phosphate, etc., mineral deposition occurs on the surface of BC sealer.[22]

Comparing the results of root canals drying with different methods and filling iRoot SP for 1 day, it was found that the root canals drying with 95% ethanol was not conducive to the attachment of iRoot SP to the root canal wall and the penetration of it into the dentin tubules, which was consistent with the results of Al-Haddad et al.[23] Under normal conditions, the dentin tubules are full of water. Excessive drying of the root canal leads to the loss of water in the dentin tubules, which is not conducive to the spreading and attachment of iRoot SP on the root canal wall and dentin tubules. As the wettability of the root canal wall was weakened, the capillary effect of the dentin tubules was reduced, and the permeability of hydrophilic materials to the dentin tubules was decreased. After soaking in SBF for 4 weeks, the minerals in the dentin tubules of the paper points drying group were also significantly more than those of the 95% ethanol drying group, indicating that 95% ethanol drying was not conducive to the biomineralizing effect of iRoot SP. iRoot SP needs to contact water molecules to initiate its hydration reaction to form hydroxyapatite. Drying the root canal with 95% ethanol caused the loss of naturally occurring water in the dentin tubules. Therefore, although ions in SBF could diffuse into the dentin tubules to provide a mineralized microenvironment, the newly formed minerals in the dentin tubules of 95% ethanol drying group were less than those of paper points drying group. Based on this, paper points were used to dry the root canals in the subsequent measurement of dentin elastic modulus and hardness and the determination of root compressive strength.

In this study, nanoindentation technique was used to test the elastic modulus and hardness of dentin. The values of elastic modulus and hardness of intertubular dentin measured by nanoindentation technique may vary due to the hydration status of dentin and the orientation of dentin tubules. The elastic modulus (17.7—21.1 GPa) and hardness (150—510 MPa) of intertubular dentin measured by Kinney in the hydrated state had certain fluctuations.[24] In this study, the elastic modulus and hardness of unfilled sample A and iRoot SP filled sample B were different at the PD-SD measuring area far from the root canal filling, which may be related to the status of different tooth samples. However, by self-comparison of the samples, it was found that there was no significant difference in the elastic modulus and hardness values of PD-SD and SD-C of the unfilled sample A, while the elastic modulus and hardness of the iRoot SP-filled sample B were significantly higher at the SD-iRoot SP area than PD-SD area. The change of dentin mechanical properties (mainly elastic modulus and hardness) is mineral dependent.[25] It has been pointed out that the elastic modulus and hardness of dentin after removing collagen in the dentin with sodium hypochlorite had no significant change, but they are significantly reduced after the acid etching with dentin.[26] Therefore, the increase of the dentin elastic modulus and hardness promoted by iRoot SP in this study could be attributed to the biomineralization effect of iRoot SP on dentin tubules.

This study verified that root canal filling with iRoot SP could enhance the fracture resistance of roots after root canal preparation through loading experiments, which is consistent with the conclusions of other studies.[14—16,27,28] Combined with the measurements of dentin elastic modulus and hardness in this study, the enhancement of the micromechanical strength of dentin by iRoot SP promoted the overall fracture resistance of the roots after root canal preparation.

In conclusion, this study demonstrated that as a hydrophilic material, excessive drying (95% ethanol drying method) is not conducive to the penetration and mineralization of the BC sealer iRoot SP into dentin tubules. iRoot SP has good biological activity, which can improve the elastic modulus and hardness of dentin and enhance the fracture resistance of the roots after root canal preparation.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

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