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Original Article

Effect of a bioactive glass-based root canal sealer on root fracture resistance ability

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Background/purpose: The root fracture resistance of endodontically treated teeth is decreased significantly, and it is more likely to fracture. This study aimed to evaluate the effect of a novel root canal sealer based on bioactive glass (BG) on root fracture resistance and explore its mechanism.

Materials and methods: The BG-based root canal sealer (BG Sealer) was prepared by mixing a kind of bioactive glass (10.8% P_2O_5, 54.2% SiO_2, 35% CaO, mol.%, named PSC), zirconia (ZrO_2) powder, sodium alginate (SA) and phosphate solution (PS). A pH meter was used to measure the pH of simulated body fluid (SBF) after immersion with BG Sealer at different time. After preparing the samples of BG sealer with a diameter of 4 mm and a height of 6 mm, the compressive strength was tested by a universal testing machine. The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to detect and analyze the mineral status of root canal systems filled with BG Sealer. The push out test was used to measure the push out bond strength of BG Sealer. The fracture resistance of root canals filled with BG Sealer was detected by the compressive loading test. Bioceramic root canal sealer iRoot SP was set as the control group.

Results: (1) Physicochemical properties: The pH value of SBF immersed with BG Sealer increased slightly up to 7.68, while the pH of SBF immersed with iRoot SP increased to 12.08. The compressive strength of the novel BG Sealer was 4.62 $\pm$ 1.70 MPa, which was lower than that of iRoot SP ($P < 0.05$). (2) Mineralization: The hydroxyapatite layers were observed on the surface of BG Sealer and iRoot SP after being immersed in SBF for 4 weeks. BG Sealer and iRoot SP were both able to penetrate into the dentin tubules, duplicate the morphology of

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root canals well, and form a layer of hydroxyapatite. (3) Adhesion to dentin: There was no significant difference between the push out bond strength of the novel BG Sealer and iRoot SP ($P > 0.05$). (4) Fracture resistance: After immersion in SBF for 4 weeks, the fracture resistance of roots filled with BG Sealer and iRoot SP was 454.16 $\pm$ 155.39 N and 445.50 $\pm$ 164.73 N, respectively, both of which were not statistically different from that of the roots unprepared and unfilled (394.07 $\pm$ 62.12 N) ($P > 0.05$), whereas higher than that of the roots prepared and unfilled (235.36 $\pm$ 83.80 N) ($P < 0.05$).
Conclusion: The novel BG Sealer has good adhesion to the root dentin, can penetrate into the dentin tubules to generate minerals, and meanwhile can improve the fracture resistance of the roots after root canal treatment. It is expected to be a bioactive root canal sealer with good clinical application prospects.

Introduction

After root canal treatment, the root fracture resistance of the tooth is decreased significantly, and thus, it is more prone to fracture, which affects the life span of the teeth.[1] Factors that affect the root fracture resistance ability of endodontically treated teeth include dehydration of dentin, mechanical preparation, chemical irrigation of roots and excessive pressure during root canal obturation.[2–4] Therefore, provided that the materials of root canal obturation boosted the mechanical properties and fracture resistance of teeth, it will have a positive impact on the retention of teeth.

The concept of "Monoblock" emphasizes the formation of an integrated structure between the root canal obturation material and the dentin of the root canal wall, that is, the obturation material and the root melt into a whole.[5] Using materials with bonding properties with dentin to form an integrated structure may enhance the fracture resistance of roots and reduce microleakage.[6,7] In recent years, it has been reported that hydrophilic bioceramic root canal sealers, such as iRoot SP, can hydrate with the water in the dentine tubules of the root canal wall to produce hydroxyapatite (HAP), which can then be firmly combined with the dentin to enhance the mechanical strength of the root.[8–11] However, it has been reported that the breaking strength of dentin is reduced by 33% when exposed to mineral trioxide aggregate (MTA) for 5 weeks. The authors proposed that this was due to the formation of calcium hydroxide in the hydration reaction of MTA, and its strong alkalinity destroys the structure of collagen in dentin.[12] Therefore, exploring neutral bioactive root canal obturation materials may improve the fracture resistance ability of tooth roots.

In a previous study, we prepared a novel bioactive glass (BG)-based root canal sealer. It has appropriate fluidity, film thickness, setting time, solubility, radiopacity and other physical and chemical properties, which meets the requirements of International Organization for Standardization (ISO) for root canal sealer. Moreover, it has good mineralization, biocompatibility and strong sealing ability to the dentin.[13] Bioactive glass is a silicate glass mainly composed of silicon dioxide (SiO_2), calcium oxide (CaO) and phosphorus pentoxide (P_2O_5), which has higher bioactivity than bioceramic materials because of its amorphous structure.[14,15] After BG is implanted into the human body, ion exchange can occur with body fluid, and the generation of hydroxyapatite can be induced through a series of biochemical reactions at the interface between BG and tissues.[16] For the type of BG used in the preparation of BG Sealer, we selected a novel neutral bioactive glass named PSC with a chemical composition of 10.8% P_2O_5, 54.2% SiO_2, 35% CaO (mol.%).[17] Compared with the classic bioactive glass 45S5, PSC components contain a high proportion of phosphorus, and hydroxyapatite can be rapidly formed when immersed in simulated body fluid (SBF), showing high bioactivity.[18] Compared with 45S5 and β-tricalcium phosphate, PSC can significantly promote the proliferation, migration and mineralization of bone marrow mesenchymal stem cells and promote their osteogenic and angiogenic differentiation.[19] In addition, PSC does not increase the pH value of the local environment and has little irritation to tissues.[17,19,20] Our study also showed that the pH value of SBF remained at a neutral pH level.[13]

Based on the excellent physical and chemical properties, sealing and mineralization ability and neutral pH value of BG Sealer, we hypothesized that BG Sealer could enhance the fracture resistance of tooth roots after root canal treatment. Therefore, by studying the adhesion to dentin and the fracture resistance of tooth roots filled with BG Sealer, this study aims to investigate whether BG Sealer can promote the fracture resistance ability of tooth roots and explore its mechanism.

Materials and methods

Components of bioactive glass-based root canal sealers

The BG-based root canal sealer, namely BG Sealer, was prepared by mixing powders and liquids, which was introduced in our previous article.[13] Seventy wt% PSC (Wooquick Technology Co., Ltd., Taizhou, China) was ground through a 400-mesh sieve (pore size, 38.5 μm), and 30 wt% zirconium oxide with a particle size of 10 nm (ZrO_2, Beijing Deke Daojin Science and Technology Co., Ltd., Beijing, China) was mixed as powder. Four mol/L Phosphate solution (PS) and sodium alginate (SA, Shanghai Aladdin Bio-Chem Technology Co., Ltd., Shanghai, China) with 1% mass volume fraction were used to prepare the liquid. Afterwards, a mixture of powder and liquid was prepared with a powder/liquid ratio (g/mL) of 1.1 to obtain BG Sealer. iRoot SP (Innovative Bioceramix, Vancouver, Canada) was set as the positive control group.

Assessment of physicochemical properties

pH value: Polytetrafluoroethylene cylinders were used to shape BG Sealer and iRoot SP into disks with 5 mm in diameter and 2 mm in thickness. Five specimens of each sealer were prepared. After curing for 24 h in an environment of 37 °C and 100% relative humidity, each specimen was immersed in 5 mL SBF incubated at 37 °C and 95% relative humidity for 1 and 4 h (h) and 1, 2, 3, 7, 14, 21 and 28 days (d). Then the pH of the solution was measured by a pH meter (PHSe3C, Shanghai Yueping Science Instrument Co., Ltd., Shanghai, China).

Compressive strength: Molds with an inner diameter of 4 mm and a height of 6 mm were fully filled with freshly prepared BG Sealer and iRoot SP and then were placed at 37 °C and 100% relative humidity. After setting for 7 days, those molds were polished utilizing wet 400 mesh sandpaper. Once polished, samples were removed from the molds, and samples with gaps or broken edges were discarded. Finally, 5 samples were prepared for BG Sealer and iRoot SP, respectively. The diameter (d) of the sealers was measured twice in the direction perpendicular to each other, and the average value was calculated to be accurate to 0.01 mm. Immediately, a universal testing machine (Instron, Boston, MA, USA) was used to measure the compressive strength along the long axis of the samples at a speed of 0.75 mm/min. The maximum load F was recorded when the sample was suddenly broken. The compressive strength is calculated according to the following formula: $C = 4F/\pi d^2$, where C is the compressive strength (MPa), F is the maximum load force (N), d is the radius of sealers (mm), and $\pi = 3.14$.

Assessment of mineralization performance

Complete single-rooted mandibular premolars with closed apical foramen were selected and cut horizontally in the middle root as 2-mm thickness dentin discs by a low-speed diamond cutting machine. A round hole with a diameter of 1 mm in the center of the root canal of each dentin disc was drilled by a round bur. All samples were soaked in 2.5% NaOCl and 17% EDTA for 3 min. Roots were randomly divided into 3 groups (3 samples per group): the unfilled group, BG Sealer group, and iRoot SP group. Each disc of the BG Sealer group and iRoot SP group was completely filled with BG Sealer and iRoot SP. Next, samples from the 3 groups were placed in an incubator at 37 °C and 100% relative humidity for 24 h, and then the samples were kept in SBF for 4 weeks, which was replaced every 2 days. The dentin discs were kept in liquid nitrogen for 24 h. Immediately after being removed, the dentin discs were broken into two halves. After drying for 3 days, the samples were observed by scanning electron microscopy (SEM, Hitachi S-4800, Tokyo, Japan) and energy dispersive X-ray spectroscopy (EDS) to determine the infiltration and mineralization in dentin tubules.

Assessment of push out bond strength

Complete maxillary central incisors with closed apical foramen were selected and embedded with self-curing resin, which was cut horizontally in the middle as dentin discs (n = 9) with a thickness of 1 mm by a low-speed diamond cutting machine (SYJ-150, Shenyang Kejing Auto-instrument Co., Ltd., Shenyang, China). Two round holes with a diameter of 0.8 mm in the dentin of each disc were drilled by a round bur. The prepared dentin discs were soaked in 2.5% NaOCl for 15 min, deionized water for 1 min, 17% EDTA for 3 min, deionized water for 1 min, 2.5% NaOCl for 1 min, and deionized water for 1 min and then dried with paper points. Each hole in one disc was filled with BG Sealer and iRoot SP, respectively. Next, the filled dentin discs were placed in an incubator at 37 °C and 100% relative humidity, and then the samples were covered with sterile gauze soaked in phosphate-buffered saline (PBS). The gauze soaked in PBS was replaced every two days and removed 1 week later. A universal testing machine (Instron, Boston, MA, USA) was used to measure the push out bonding strength. The thrust head with a diameter of 0.6 mm was pushed out perpendicular to the dentin disc at a speed of 0.5 mm/min, and then the maximum pressure value (F) was recorded when the filling material suddenly dislocated. The push out bond strength is calculated according to the following formula: $P=F/(\pi dh)$, where P is the push out bond strength (MPa), F is the maximum load force (N), d is the radius of sealers (0.8 mm), and h is the thickness of the dentin disc (1 mm).

Assessment of fracture resistance

Complete single-rooted mandibular premolars with closed apical foramen were chosen to assess the fracture resistance of teeth. Roots with a height of 12 mm were cut and retained by the low-speed diamond cutting machine. Then, we measured the buccolingual diameters of the roots, as well as the mesiodistal diameters, and divided them into large, medium and small groups. Work length was determined at 1 mm short from the root apex. With the aid of EDTA, roots were prepared by the M3 nickel-titanium file (United Dental, Shanghai, China) up to size 30/.04. After preparation, the canals were irrigated with 2 mL 2.5% NaOCl, 2 mL 17% EDTA solution and 2 mL deionized water, which was followed by ultrasonic irrigation, and dried with paper points. Roots were randomly divided into 4 groups (9 samples per group): negative control group (unprepared and unfilled), positive control group (prepared and unfilled), BG Sealer group, and iRoot SP group. BG Sealer was freshly prepared and delivered into the root canal until 1 mm short from the canal orifice. iRoot SP was inserted into the root canal utilizing its own injection needle until 1 mm short from the canal orifice. Temporary restorative material (Ceivitron) was used to seal the root canal. All samples were placed at 37 °C and 100% relative humidity for 1 week and then soaked in SBF for 4 weeks, which was replaced every 2 days.

All samples were embedded in a plastic tube with an inner diameter of 20 mm and a height of 20 mm along the long axis of the root, and roots with a height of 9 mm above the surface of the resin were exposed. Roots were vertically loaded by a 3-mm diameter hemispherical tip at a speed of 1 mm/min until fracture using a universal testing machine. The maximum load to fracture was recorded as the fracture resistance.

Statistical analysis

The quantitative data are expressed as the mean ± standard deviation, and the experimental data were analyzed by SPSS 24.0 software (IBM, Chicago, IL, USA). The data of compressive strength were analyzed by independent sample T tests. The data of push out bond strength were analyzed by paired samples T test, and the data of fracture resistance were analyzed by homogeneity of variance test and one-way variances (ANOVA). $P < 0.05$ indicated a statistically significant difference.

Results

Physicochemical properties of BG sealer

pH value of sealers in SBF

The pH values of SBF immersed with BG Sealer and iRoot SP were shown in Fig. 1. The pH value of SBF immersed with BG Sealer increased slightly up to 7.68 from 1 h to 28 days, whereas the pH value of SBF immersed with iRoot SP raised rapidly from 7.82 at 1 h to 12.08 at 14 d and then tended to be stable.

Compressive strength of sealers

As shown in Table 1, the compressive strength of BG Sealer and iRoot SP was 4.62 ± 1.70 MPa and 8.58 ± 2.42 MPa, respectively, and there was a significant difference between them ($P < 0.05$).

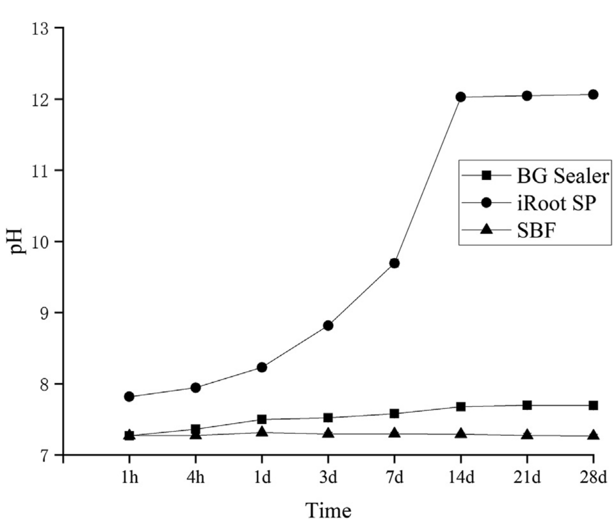

Figure 1 pH value change of SBF immersed with root canal sealers at different time.

Table 1 Compressive strength of BG Sealer and iRoot SP.

	N	Compressive strength (MPa)	t	P value
BG Sealer	5	4.62 (±1.70)	−2.991	0.017
iRoot SP	5	8.58 (±2.42)		

Abbreviations: N, number; MPa, megapascal.

Mineralization of root canals filled with BG Sealer

Fig. 2 a-c showed that collagen in the dentin tubules was exposed in the unfilled group, whereas BG Sealer and iRoot SP penetrated into the dentin tubule and fully replaced the shape of dentin tubule, and a sheet or globular mineral formation was observed on the surface (Fig. 2 d-g, h-k). EDS was used to further analyzed the mineral elements on the surface of materials penetrating into the dentin tubules, where the minerals generated by BG Sealer and iRoot SP are composed of Ca, P, O, Si, and with Ca/P ratios of 1.95 and 1.88, respectively. These results suggest that BG Sealer can penetrate into the dentin tubule, replicate the morphology of dentin tubule, and form apatite minerals on the surfaces.

Push out bond strength of sealeres filling in root canals

The push out bond strength of BG Sealer and iRoot SP was shown in Table 2. Results indicate that there is no statistical difference between BG Sealer and iRoot SP ($P > 0.05$).

Fracture resistance of roots filled with BG Sealer

As represented in Fig. 3, after immersion in SBF for 4 weeks, there was no statistical difference between the fracture resistance of roots filled with BG Sealer and iRoot SP (454.16 ± 155.39 N, 445.50 ± 164.73 N, $P > 0.05$), both of which were not statistically different from that of the roots unprepared and unfilled (394.07 ± 62.12 N) ($P > 0.05$), whereas higher than that of the roots prepared and unfilled (235.36 ± 83.80 N) ($P < 0.05$). Results suggested that roots filled with BG Sealer soaked in SBF for 4 weeks could enhance the fracture resistance of roots after root canal preparation.

Discussion

The results of this study showed that the novel BG-based root canal sealer had good adhesion to the root dentin, could penetrate into the dentine tubules to generate minerals, and improved the fracture resistance of the roots after root canal treatment.

In terms of the material composition of BG sealer, the bioactive glass PSC synthesized with phytic acid as a precursor was selected in this study. It is prepared by the sol–gel method with a low heat treatment temperature and large specific surface area. PSC has high phosphorus content and can react with PS to rapidly generate hydroxyapatite.[17–20] When traditional BG or other calcium silicate material is in contact with tissue fluid, due to the release of

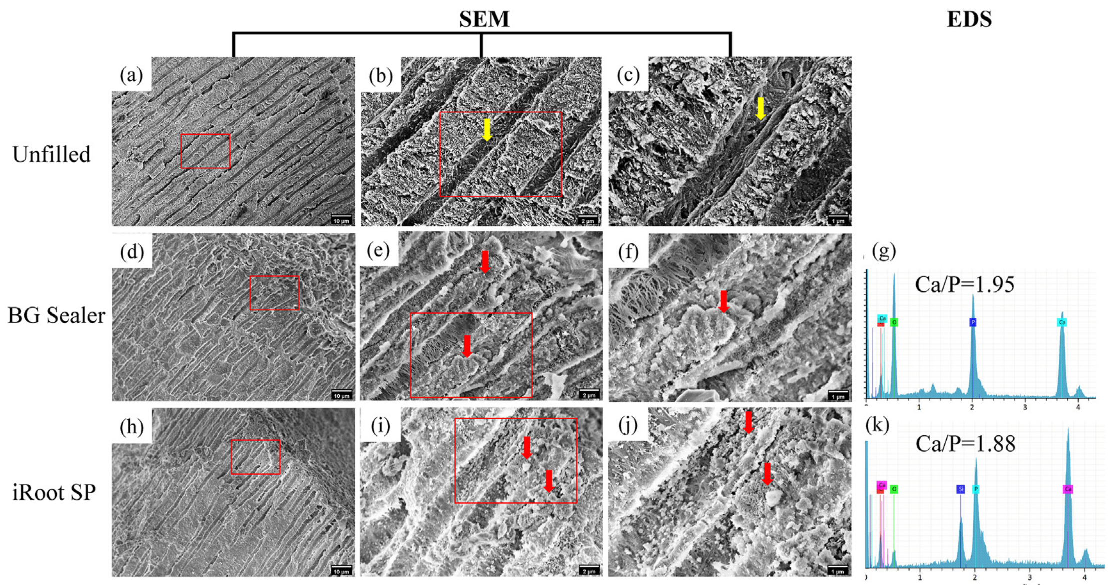

Figure 2 Photomicrographs of penetration and mineral formation in dentin tubules by SEM. The second column was the enlargement of the red box in the first column, and the third column was the enlargement of the red box in the second column. The yellow arrow showed the exposure of collagen fiber (b, c). The red arrow showed that sealers penetrated into the dentin tubule, replicating the morphology of the root canal, and mineral deposits were formed on the surface (e, f, i, j). EDS showed that the minerals are deposited (g, k). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2 Push out bond strength of BG Sealer and iRoot SP.

	N	Push out bond strength (MPa)	t	*P* value
BG Sealer	9	3.41 (±2.40)	−0.789	0.453
iRoot SP	9	4.47 (±1.92)		

Abbreviations: N, number; MPa, megapascal.

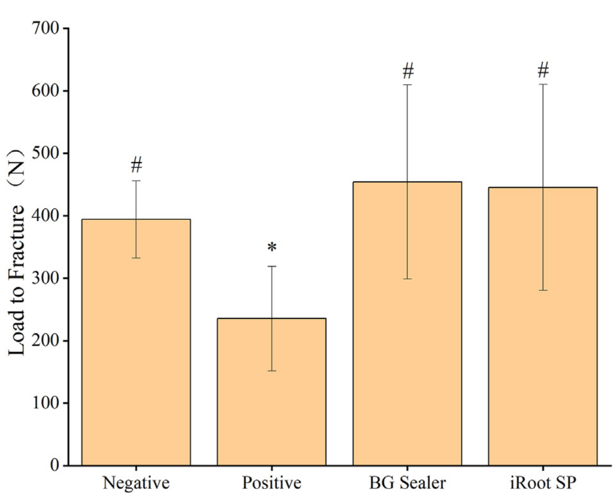

Figure 3 Loads to fracture of roots with or without being filled with sealers (n = 9). * represented statistically significant difference compared with the negative control group, $P < 0.05$. # represented statistically significant difference compared with the positive control group, $P < 0.05$.

Ca^{2+} from the material and the exchange with H^+ in the tissue fluid, the pH of the surrounding medium increases, which will produce strong irritation to tissue cells in the initial stage.[21] In this study, we also observed that the pH value of SBF immersed with iRoot SP continued to rise to approximately 12. The pH value of SBF immersed with BG sealer remained at a neutral pH level, because PSC components do not contain sodium and have a relatively high phosphorus content. After contacting with SBF, the acidic phosphorus containing substances released are conducive to compensating for the pH rise caused by the exchange of Ca^{2+} and H^+.[22]

Dentin is a biomineralized tissue mainly composed of type I collagen, highly ordered hydroxyapatite and a small amount of noncollagenous protein. Inorganic minerals are orderly arranged inside and outside type I collagen fibers to form intrafibrillar mineralization and extrafibrillar mineralization, which jointly protect collagen fibers from hydrolysis.[23] The effective combination of minerals and collagen plays a major role in the mechanical properties of dentin.[24] Li found that pH neutral bioactive glass PSC could promote the extrafibrillar mineralization of type I collagen, and PSC combined with polyacrylic acid (PAA) could promote both extrafibrillar mineralization and intrafibrillar mineralization.[25] Alkaline calcium hydroxide will be formed in the hydration reaction of iRoot SP, and a previous study reported that alkaline materials could cause conformational changes in collagen proteins.[12] The compressive strength of BG Sealer prepared in this study was lower than that of iRoot SP, which was expected to result in lower fracture resistance ability of it. However, the results revealed that there were no statistically significant differences in the push out bond strength and fracture resistance of roots between the two sealers, possibly due to BG sealer had no pH change in the tissue and could promote the mineralization of dentin collagen.

Hench et al. believed that the mineral ions released by BG in contact with body fluids and the formation of HAP were the main reasons for its biological activity. When BG is in contact with body fluid, positive ions such as Ca^{2+} exchange with H^+ in body fluid, forming Si-OH on the surface of BG. Si-OH polycondensates on the BG surface to form a silicone gel layer. PO_4^{3-} and Ca^{2+} migrate to the silicone gel layer to form an amorphous Ca-P layer, and the Ca-P layer gradually accumulates and crystallizes from an amorphous state to form a HAP layer.[16] In this study, SEM and EDS were used to observe the penetration and mineralization of BG Sealer into dentin tubules after filling root canals and soaking in SBF for 4 weeks. The results showed that BG Sealer was able to penetrate into the dentin tubules well and generate apatite minerals, indicating that BG sealer could use calcium and phosphorus ions to form a mineralized layer after penetrating into the dentin tubules. This also explains the strong push out bond strength between BG Sealer and dentin and the enhanced fracture resistance of roots.

Previous studies have proven that the use of materials with adhesive properties to dentin can form an integrated structure between dentin and materials, which may improve the sealing of tooth roots and enhance the fracture resistance.[6,7] The study found that there was a certain correlation between the push out bond strength and the sealing and fracture resistance of the root.[26–28] Therefore, this study used the push out bond strength test to detect the adhesion between BG Sealer and the root canal, and indirectly reflect the effect of BG Sealer on the fracture resistance of the root. In addition to the chemical bonding between the root canal sealer and dentin, the "bond strength" measured by the push out bond strength test also has a certain friction force. Although this method cannot completely simulate the clinical situation,[29] it is still the best test method for the adhesion of root canal sealers.[30,31] The push out bond strength test used in this study refers to the improved method proposed by Scelza et al. This method can be standardized and has low technical sensitivity. Due to the balance of sample baseline, the influence of confounding factors such as tooth age, mineralization degree and hardness can be ignored.[32,33] In this study, no statistically significant difference was found between the novel BG Sealer and iRoot SP ($P > 0.05$) in push out bond strength, while iRoot SP was proven to be the leader of calcium-silicon-based root canal sealers.[34]

In this study, the fracture resistance of roots filled with different root canal sealers was detected by the compressive loading method, and the roots were immersed in SBF for 4 weeks to simulate the situation in vivo. The reason that only the root canal sealer but no gutta percha was filled into the root canals in this experiment was to better observe the effect of sealer itself on the fracture resistance of roots. On the one hand, it was to avoid the possible uneven thickness of sealer between samples after the use of gutta percha to make samples more comparable. On the other hand, the compressive strength of gutta percha is low that it could hardly change the root fracture resistance and some studies even used the root canals filled with gutta percha without the sealer as the positive control group.[35] Sungur et al. has reported that iRoot SP with or without gutta-percha showed similar root fracture resistance.[36] The results of this study showed that the average fracture resistance of the roots prepared and unfilled was 235.36 N, which was less than that of unprepared and unfilled roots (394.07 N). The difference between the two materials was statistically significant ($P < 0.05$), which could be interpreted that the root canal preparation process led to the reduction of root mechanical properties. However, after filling the root canal with iRoot SP, the root fracture resistance increased to 445.50 N, which was significantly higher than that in the prepared and unfilled group ($P < 0.05$). This was consistent with the research results of Sağsen, Hegde, Sungur and Celikten.[8,10,11,36] In this study, the fracture resistance of roots filled with BG Sealer increased to 454.16 N which was no significant difference compared with iRoot SP ($P > 0.05$). Therefore, we think BG Sealer has been shown to improve root fracture resistance.

Although ISO has no standard requirement for the compressive strength of root canal sealers, the use of materials with high compressive strength and bonding with teeth may potentially improve the fracture resistance of roots.[5] Therefore, in the future, the composition of BG Sealer will be further improved to increase its own compressive strength, in order to better enhance the fracture resistance of roots after root canal treatment.

In conclusion, the novel BG Sealer has good adhesion to the root dentin, can penetrate into the dentin tubules to generate minerals, and can improve the fracture resistance of the roots after root canal treatment. It is expected to be a bioactive root canal sealer with good clinical application prospects.

Declaration of competing interest

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

Acknowledgements

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