Evaluation of selected properties of a new root repair cement containing surface pre-reacted glass ionomer fillers.

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Abstract

Objective: This study evaluated selected properties of a prototype root repair cement containing surface pre-reacted glass ionomer fillers (S-PRG) in comparison to mineral trioxide aggregate (MTA) and intermediate restorative material (IRM).

Materials and methods: The antibacterial effect of S-PRG, MTA and IRM cements were tested against *Porphyromonas gingivalis* and *Enterococcus faecalis* after one and three-days of aging of the cements. Set cements were immersed in distilled water for 4 hours to 28 days and ion releasing ability was evaluated. Initial and final setting times of all cements were evaluated using Gilmore needles. The push-out bond strength between radicular dentin and all cements was tested at different levels of the roots.

Results: S-PRG and IRM cements but not MTA cement demonstrated significant antibacterial effect against *Porphyromonas gingivalis*. All types of cements exhibited significant antibacterial effect against *Enterococcus faecalis* without being able to eliminate the bacterium. S-PRG cement provided continuous release of fluoride, strontium, boron, sodium, aluminum and zinc throughout all tested time points. Both initial and final setting times were significantly shorter for S-PRG and IRM cements in comparison to MTA. The push-out bond strength was significantly lower for S-PRG cement in comparison to MTA and IRM at coronal and middle levels of the roots.

Conclusions: S-PRG cement demonstrated significant antibacterial effects against endodontic pathogens, multiple ion releasing ability, relatively short setting time and low bonding strength.

Clinical relevance: S-PRG cement can be used as a one-visit root repair material with promising antibacterial properties and ion releasing capacity.

Keywords: IRM, Ion release, MTA, setting time, Push-out bond strength, S-PRG fillers

Introduction

Various endodontic repair cements have been used in different procedures such as root-end fillings during endodontic microsurgical techniques, perforation repairs, and artificial apical barriers in necrotic teeth with immature roots. The most widely used root-end filling material is a mineral trioxide aggregate (MTA), which is a calcium-silicate cement. MTA possesses several advantages, such as good sealing ability [1], acceptable mechanical properties [2], biocompatibility [3], and some antibacterial properties [4, 5]. However, the main drawbacks of MTA are its high cost, low radiopacity, difficult manipulation and long setting time [2, 6]. The 3-4 hour setting time of MTA might increase the risk of wash-out of the freshly applied cement in excessively wet environments [7]. Other dental materials, such as dental amalgam, intermediate restorative material (IRM), and Super EBA have also been used as root-end filling materials. Indeed, the clinical success rate of MTA root-end filling was found to be comparable to IRM [8, 9] and Super-EBA [10].

Recently, various dental materials containing surface pre-reacted glass-ionomer fillers (S-PRG) were introduced by Shofu Inc. [11]. Multiple S-PRG-based dental materials are commercially available and have been collectively categorized as Giomer (Glass ionomer + polymer) [12]. The S-PRG fillers are prepared by the initiation of an acid-base reaction between fluoroboroaluminosilicate glass and aqueous polyacrylic acid. These bioactive S-PRG particles are thought to promote remineralization [13] and induce antibacterial effects [14, 15] through the release of multiple ions such as fluoride, strontium, sodium, boron, aluminum and silicon [16, 17]. The aim of this study was to evaluate selected antibacterial, physiochemical and bonding properties of a prototype S-PRG filler-based root repair cement in comparison to commercially available materials used as root repair cements, namely MTA and IRM.

Materials and methods

Root cement materials

A prototype of S-PRG filler containing root repair cement was provided by Shofu (S-PRG root cement, Lot No. 14315, SHOFU, Kyoto, Japan). Furthermore, MTA (ProRoot MTA, Lot No. 201404-01, Dentsply Tulsa, TN, USA) and IRM (Intermediate Restorative Material, Lot No. 130605, Dentsply Caulk, DE, USA) were also used in this study.

Bacterial strains and culture conditions

Porphyromonas gingivalis (ATCC 33277) and *Enterococcus faecalis* (ATCC 29212) were selected because they are the most frequent endodontic pathogens present in primary [18, 19] and secondary root canal infections [20, 21], respectively. The selected bacteria were individually grown on blood agar plates (CDC; BioMerieux, Durham, NC, USA). Colonies of both bacteria were then suspended separately in brain-heart infusion (BHI) broth supplemented with 5 g yeast extract/L and 5% v/v vitamin K+ hemin (BHI-YE; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to make suspensions of 1×10^6 CFU/mL of *E. faecalis* or *P. gingivalis* after 24 h incubation at 37ºC with 5% CO_2. Gas-generating sachets (Gas-Pak EZ; Becton) were used to create the required anaerobic environment for *P. gingivalis*.

Preparation of cement discs and antibacterial testing

Each of the three tested cements was mixed according to the manufacturer's instructions and placed into polyvinyl molds with a 4 mm diameter and 2 mm height to make cement discs. Then, the discs were incubated for 1 or 3 days at 37ºC and approximately 100% relative humidity. After each time point, the discs were removed, immersed in 70% ethanol for 10 seconds as described in previous studies [22, 23] and flamed dry prior to use. Discs were individually immersed in glass tubes containing 5 mL of *E. faecalis* or *P. gingivalis* bacterial

suspension and incubated for 48 hours. Glass tubes containing the same volume of bacterial suspension without cement discs were also incubated as controls. Furthermore, pilot studies were conducted to confirm the absence of any turbidity after short term immersion of various cements into bacteria-free culture media. After the 48 hours of incubation, glass tubes with or without cement discs were vortexed for 10 seconds. Then, 200 µl of bacterial suspension from each tube was transferred into a microtiter plate (Fisher Scientific Inc., Fair Lawn, New Jersey, USA) and the optical density absorbance was read at 595 nm using a spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA).

Three independent experiments were performed in triplicate. Thus, a total of 9 discs from each type of cement were used for each bacterium at each time point. The percentage of bacterial growth from each cement disc was calculated according to the following equation: Bacterial growth (%) = (experimental absorbance value)/(control absorbance value) × 100.

Ion-releasing abilities

Plastic molds of 12 mm internal diameter and 4 mm height were filled with fresh cement pastes (n = 3 for each material), compacted with a stainless steel spatula, and stored at a relative humidity of 95% at 37ºC for 4 h. The samples were then taken out of the molds, individually immersed in 10 mL of laboratory grade distilled water (Carolina Biological, Burlington, NC, USA) and stored at 37ºC. The exposed surface area of each sample was 264 mm^2 (upper and lateral surfaces). The solutions containing the samples were collected at pre-determined end-point times (4 and 24 hours, and 3, 7, 14, and 28 days) and each sample was moved to a new 10 mL of distilled water at the beginning of each period. Containers with 10 mL of distilled water and no cement were also used to insure that the detected chemical elements in all solutions were actually leached from the cements and not already present in the water. The solutions collected at

each time point were utilized for pH measurement and elemental analyses. The ions analyzed were calcium, fluoride, strontium, sodium, zinc, aluminum, and boron.

The pH measurements were performed with a pH Meter and probe (Accumet, Fisher Pittsburgh, PA, USA). The pH probe was calibrated using standard solutions with pH values of 4, 7, and 10. The calcium concentration in the solutions was analyzed using flame atomic absorption spectrometry (AAnalyst 200; Perkin-Elmer, Shelton, CT, USA) at 422.7 nm. The tested solutions were diluted with lanthanum chloride solution and calibration was performed using standard calcium solutions (1.25–5.00 μg Ca/mL). Fluoride determination in the solutions was conducted utilizing a fluoride ion specific electrode (Orion 96-09; Thermo Electron, Beverly, MA,USA) connected to an ion meter (Orion Research Inc., Boston, MA, USA). Standard solutions (0.01–100.00 μg F/mL) were used for calibration. The standard solutions and test solutions were prepared with 1 mL of total ionic strength adjustment buffer II (TISAB II, Sigma Aldrich, St. Louis, MO, USA) to 1 mL of standard/test solution. The readings were expressed in millivolts (mV) and transformed to ppm through linear regression of the calibration curve.

The concentrations of the remaining elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; 6100; Perkin Elmer, Norwalk,CT, USA). The conditions used for the analysis were 27 MHz and 1300 W of power from a radiofrequency generator, a plasma argon gas flow of 15 L/min, an auxiliary argon gas flow of 0.8 L/min, and carrier argon gas flow of 0.2 L/min. The ICP-AES was calibrated using a four-point calibration method with standard solutions and the detection limits of analyzed elements ranged between 0.004-0.06 ppm. The cumulative releases of all elements were calculated by summing the non-cumulative release over time and the obtained values were expressed as ppm.

Setting time evaluation

The initial and final setting times of the three cements were tested according to ADA specifications [24] using Gilmore needles (Humboldt MFG., Norridge, IL, USA). The Gilmore needle used to determine initial setting time was 113.4 g with a 2.12 mm tip diameter. The Gilmore needle used to evaluate final setting time was 453.6 g with a 1.06 mm tip diameter. The setting times were calculated as the time elapsed (min) between the mixing of the powder cement with liquid and the point at which Gilmore needle indentation ceased to be visible on the surface of the materials. A large number of cement samples were initially used to find the approximate initial and final setting times. Molds of 10 mm in internal diameter and 2 mm in thickness were filled with fresh cement pastes and stored at 37 ±1 ºC and 95% relative humidity. As the initial or final setting times approached, the samples were tested every minute inside the incubator to determine the exact Gilmore setting time. Gilmore needles were place inside the incubator at least 1 hour before the commencement of testing. The initial and final setting time evaluations were performed in triplicate for each material.

Push-out bond strength test

The push-out bond strength test was performed as described in a previous study [25]. Intact single rooted human premolars (n=30) with minimum apical curvature (less than 5º) were selected for this study after obtaining local IRB approval. The teeth were stored at 4 °C in 0.1% thymol solution and used within 6 months after extraction. All teeth were horizontally decoronated at the level of 0.5 mm radicular to the facial cementoenamel junction using a water cooled low-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA) generating 15±1 mm long

roots. The working length of each root was determined by visualizing the tip of a size 15 K-file (Brasseler, Savannah, GA, USA) extending beyond the apical foramen and subtracting 1 mm from that length of the file. The root canals were mechanically prepared using EndoSequence 0.06 taper rotary instruments (Brasseler) to a master apical size 55 file. One mL of 5.25% sodium hypochlorite irrigation was used between sequential files. Additionally, each canal was finally rinsed with 1 mL of 5.25% sodium hypochlorite for 1 min followed by 1 mL of 17% EDTA for 1 min and 1 mL of sterile water for 1 min using a 27-gauge needle. The roots were then randomly divided into three treatment groups according to the obturating cement used. S-PRG, MTA, and IRM cements were prepared according to the manufacturers' instructions and manually tamped into the root canals in their respective groups using hand pluggers. The coronal 2 mm of each root was filled with Cavit (3M ESPE, St Paul, MN, USA) and each filled root was radiographed mesiodistally and buccolingually to confirm the three-dimensional obturation. Roots were then wrapped in pieces of gauze soaked in saline (pH = 7.2) and incubated at 37 ºC for 7 days to allow complete setting of MTA.

After seven days, three 2-mm thick cylinders were cross-sectioned from each root at the coronal, middle, and apical levels using a water-cooled diamond saw. The coronal and apical root canal diameter and the thickness of each root cylinder were measured to the nearest 0.01 mm utilizing a digital caliper. The area of adhesion between the cement and each root cylinder was estimated according to the following equation:

$$\text{Adhesion surface area } (mm^2) = \left(\frac{D1 + D2}{2}\right) \times \pi \times h$$

Where D1 and D2 were the greater and lesser canal diameters, respectively, π was the constant 3.14 and h was the thickness of the obturated root cylinder.

The amount of force required to displace the obturation material from each root cylinder was measured using a universal testing machine (Sintech Renew 1123, MTS, Eden Prairie, MN, USA). Each root cylinder was fixed on the center of a metal disc that had a central hole with the coronal side facing away from the point of load application. The central hole within the metal disc was slightly larger than the coronal root cylinder diameter to support the root cylinder and allow easy dislodgment of the obturating cement. Cylindrical metal plungers (9, 7, and 5.5 mm in diameter for coronal, middle and apical root cylinders, respectively) attached to the loading cell were used to apply compressive force on the obturating cement at a crosshead speed of 0.5 mm/min. Each metal plunger had a clearance of at least 0.15 mm from the root wall margins. The maximum dislodgement force of the obturation material was recorded in Newtons and the push-out bond strength (MPa) was calculated for each sample using the following equation: Push-out bond strength (MPa) = the dislodgment force (N) / adhesion surface area (mm^2).

After the push out test, the samples were examined with stereomicroscopy (Nikon UM-2, Tokyo, Japan) at 40× magnification to categorize the failure pattern according to the following classification: (1) adhesive (between dentine and the obturation cement), (2) cohesive (within the obturation cement), or (3) mixed.

Statistical analyses

All data were checked for normality using the Kolmogorov-Smirnov test and natural logarithm transformations were conducted when necessary to satisfy the normality assumptions. Mixed model ANOVA followed by Fisher protected least significant differences were used to statistically analyze data from antibacterial activity, ion release and push-out bond strength assays. Additionally, one-way ANOVA followed by Fisher protected least significant differences was used to statistically analyze data from both initial and final setting times of different

cements. Cumulative logit generalized estimating equations (GEE) model including the fixed effects of root location and cement material was used to statistically analyze data from mode of failure after push-out bond strength test. Statistically significant differences were established when $p < 0.05$.

Results

Antibacterial activity against *P. gingivalis*

Both S-PRG and IRM cements caused a significant reduction in the percentage of bacterial growth compared to the control ($p<0.005$) at both time points (Table 1). On the other hand, MTA demonstrated a significantly higher percentage of bacterial growth compared to the control after one day of cement preparation ($p<0.0001$). S-PRG provided significantly more reduction in bacterial growth compared to IRM and MTA ($p<0.0001$) at both time points. Additionally, IRM provided a significantly higher reduction in bacterial growth compared to MTA ($p<0.0001$) at both time points.

Antibacterial activity against *E. faecalis*

All tested cements caused a significant reduction in the percentage of bacterial growth compared to the control at both time points ($p<0.0001$; Table 1). Furthermore, MTA demonstrated significantly higher reduction in bacterial growth compared to S-PRG and IRM ($p<0.0001$) at both time points. Additionally, S-PRG provided significantly higher reduction in bacterial growth compared to IRM ($p<0.0001$) at both time points. The time factor did not have a significant effect on the percentage of bacterial growth.

Ion releasing abilities

The non-cumulative and the cumulative releases of all tested ions in the leachates as well as pH are shown in Table 2. S-PRG cement showed the ability to release 6 different ions throughout the soaking period. S-PRG cement provided an initial burst of boron, zinc and sodium followed by continuous ion release throughout the experiment. Both fluoride and aluminum reached their peak release from S-PRG cement after three days while the release of strontium showed gradual and continuous increase throughout the experiment. MTA cement demonstrated an initial burst of calcium release after 4 hours soaking followed by constant release of calcium at all endpoint times. Sodium, aluminum, and strontium released by S-PRG cement were significantly higher than the same ions released from MTA and IRM at the majority of soaking times ($p<0.0001$). S-PRG release of zinc was significantly higher than Zn released from IRM after 4 hours and 1 day ($p<0.0001$) of water soaking.

S-PRG maintained the pH of the distilled water unchanged throughout the soaking period. IRM also maintained the pH of the distilled water unchanged until the two week time point but it showed significant acidifying effect compared to the control at the four week time point. Furthermore, S-PRG provided significantly higher pH of the distilled water compared to IRM at 4 hours ($p<0.0039$), 1 week ($p<0.02$) and 4 week ($p<0.0002$) time points. Additionally, the pH in the presence of MTA cement was significantly higher than that of the control and all other materials at the majority of tested endpoints ($p<0.03$- $p<0.0002$).

Setting time

The effect of type of cement was significant for both initial and final setting times ($p< 0.0001$). Both initial and final setting times were significantly shorter for IRM and S-PRG

cements than for MTA ($p < 0.0001$) (Figures 1A and B). However, no significant difference in initial and final setting times was observed between IRM and S-PRG.

Push-out bond strength

The location-by-type of cement interaction was significant for push-out bond strength ($p<0.0001$). Therefore the pairwise differences of cement materials were performed at each root third (Table 3). MTA had significantly higher bond strength at the coronal and middle parts of the root compared to IRM and S-PRG cements ($p<0.0001$). Furthermore, IRM had significantly higher bond strength compared to S-PRG ($p<0.001$). The bond strength of IRM at the apical part of the root was significantly higher compared to that of MTA and S-PRG ($p<0.0001$). The push-out bond strength of MTA was significantly lower in the apical third of the roots compared to both coronal and middle thirds ($p<0.0001$). On the other hand, the push out bond strength of IRM was significant higher in the apical third of the roots compared to coronal third ($p=0.02$). Modes of failure were predominately cohesive or mixed except for S-PRG cement on the coronal and middle thirds of the roots, in which adhesive or mixed failure occurred in the majority of the samples. However, the interaction between type of cement used and level of roots was not significant for mode of failure. Furthermore, no significant difference was detected in mode of failure between the three types of cement at all root levels.

Discussion

The strategy of using various bioactive glass particles in dental applications to improve dentine remineralization has been proposed for decades [26, 27]. However, the incorporation of

bioactive glass fillers into relatively bioinert materials has gained popularity in recent years as an attempt to achieve more specific biological responses such as inducing antibacterial action [28] or promoting a particular cell response [29]. The antibacterial test used in this study was a modified direct contact test, which is a common antibacterial test used in the endodontic literature to test the antibacterial properties of root cements [4] and sealers [30]. However, the main limitation of the regular direct contact test is that it does not allow evaluation of microorganisms under biofilm conditions [5] because any attempt to disrupt the bacterial biofilm on cement surfaces would lead to crushing of the unset cement samples. Therefore, the modified direct contact test was performed on set cement samples in the current study in order to be able to vortex the tested cement samples and evaluate the bacterial biofilm grown on the specimen surfaces as well as planktonic bacteria around the samples. Future studies aiming to investigate the antibacterial effects of these cements against bacterial biofilms rather than planktonic bacteria are necessary to confirm the antibacterial finding of the current study.

In the present study, S-PRG cement showed significant reduction in *P. gingivalis* growth ranging between 95-99%. The ability of S-PRG cement to nearly eradicate *P. gingivalis* bacteria can be explained by considerable ion release with antibacterial activity such as boron, strontium, and fluoride. The antimicrobial ability of boron-containing compounds has been a subject of interest in recent years [31, 32]. Indeed, boron-based antibacterial therapeutics were suggested to possess strong antibacterial activity against gram-negative infections due to their ability to inhibit various bacterial enzymes [33, 34]. The current study showed that S-PRG cement was able to release significant amounts of boron throughout all time points. The strong antibacterial effect of S-PRG cement against gram-negative *P. gingivalis* as reported in this study may indicate a potential efficient use of this new root repair material in primary endodontic infections due to the

presence of higher level of gram-negative bacteria compared to secondary endodontic infections [35, 36]. However, further studies are required to evaluate the antibacterial properties of S-PRG cement against other gram-negative endodontic pathogens. It is also worth noting that the ability of S-PRG cement to release both fluoride and strontium may also play an additional antibacterial role as the combination of these elements was suggested to have significant antibacterial action [37].

Our study also demonstrated that IRM caused a significant reduction in *P. gingivalis* growth and this reduction was significantly higher for IRM after three days aging. However, the IRM reduction in *P. ginigvalis* growth ranged between 11-23%, which was significantly lower than the bacterial growth reduction caused by S-PRG cement. A previous study also found that a zinc-oxide eugenol based sealer had significant antibacterial effect against *P. gingivalis* [30]. The antimicrobial effect of IRM could be explained by the antibacterial ability of eugenol released from IRM by progressive hydrolysis [38]. No antibacterial effect of MTA against *P. ginigivalis* was observed in the current study, which is consistent with previous studies [30, 39]. All tested cements caused significant but limited reduction on *E. faecalis* growth ranging from 2-15% compared to control. Furthermore, MTA provided significantly more reduction of *E. faecalis* growth compared to IRM and S-PRG cements. The ability of MTA to create an alkaline environment may explain its antibacterial effect against *E. faecalis* [6]. Previous studies have also demonstrated a significant antibacterial effect of MTA, IRM and glass ionomer like material against *E. faecalis* without being able to totally eliminate the bacterium [4, 5, 40].

Our study demonstrated that S-PRG released 6 different types of ions throughout all tested time points, which is consistent with previous studies conducted on various S-PRG based materials [16, 41]. In addition to the expected antibacterial action of some of the ions released

by S-PRG, ions like fluoride and strontium may play an important role in apatite formation and stability. Previous studies also showed that the combination of fluoride and strontium caused a synergistic remineralization effect [42, 43]. Ions released from the S-PRG filler were suggested to promote apatite induction [17], increase dentine remineralization [44] and improve dentine acid resistance [45]. Furthermore, S-PRG based root canal sealer was able to establish a superficial surface layer in root canal dentine that is rich with fluoride, strontium, and silicon [45]. This study also confirms the findings of previous studies that showed continuous release of calcium ions from MTA [2, 46]. On the other hand, no calcium release was detected from S-PRG cement. These findings may indicate distinct differences in the potential mechanism of action of MTA versus S-PRG cement in both antibacterial and remineralization effects. The MTA mode of action is mainly facilitated by the continuous leaching of calcium ions and the increase in local pH during hydration reaction while the S-PRG mode of action is mainly derived by the release of multiple ions other than calcium and maintaining a relatively neutral pH of the local environment.

One of the concerns related to the use of S-PRG root cement is the potential cytotoxic effect of some of the ions released, specifically fluoride. However, the cytotoxicity of fluoride is pH dependent [47, 48] and previous studies have shown that fluoride is cytotoxic in acidic pH but has minimum cytotoxic effect in neutral pH [47, 48]. Therefore, the ability of S-PRG cement to maintain the pH of the soaking water unchanged throughout the soaking period might be helpful in minimizing the cytotoxic effect of fluoride. A recent study suggested that S-PRG filler containing composite was significantly less cytotoxic than ceramic reinforced glass ionomer cement, conventional glass ionomer cement and resin composite [49]. Nevertheless, further

studies are required to investigate the biocompatibility of S-PRG root repair cements on both cellular and histological levels.

The initial and final setting times of S-PRG reported in this study were 11 and 30 minutes, respectively. This is significantly shorter than MTA initial and final setting times, which were 35 and 200 minutes, respectively. The final setting time of MTA reported in this study is consistent with that reported in the literature, which ranged between 165-250 minutes [2, 50, 51]. The relatively short setting times of S-PRG cement may provide for a reasonable one-visit root repair material, which is not possible with the ProRoot MTA. Cements with traditional acid-base reaction systems such as S-PRG and IRM are expected to set faster than calcium-silicate cements that rely primarily on hydration reactions for setting, which is usually slow [52]. Further studies are required to investigate other variables of S-PRG cement that are related to the setting process such as dimensional stability, solubility and expansion.

Various chemical compositions of root repair materials may lead to different interaction between these materials and radicular dentine. Therefore, push out bond strength was used in the current study to explore the dislocation resistance of various root materials. It is the most reliable mechanical test that can rank the dislodgment resistance of various endodontic materials applied to dentine such as root canal sealers, root repair materials and intraradicular posts [53]. The push-out bond strength was significantly higher for MTA and IRM compared to S-PRG cement at both coronal and middle thirds of the roots. The relatively fast expansion [54] and biomineralization [55] abilities of MTA may improve the mechanical retention of MTA and explain its superior bonding strength on the coronal and middle part of the roots compared to other tested cements. Furthermore, the low push-out bond strength of S-PRG cement might be attributed to the relatively short storage time before evaluation of the bond strength in the current

study (7 days), which was selected because it is the common storage time used in the vast majority of studies investigating the push-out bond strength of calcium silicate root cements [25, 56]. Fluoride, strontium and silicon ions released from a prototype S-PRG-based root canal sealer were suggested to incorporate into root dentine significantly deeper after one and three months of application compared to one week application [41]. The bond strength of MTA at the apical part of the roots showed a severe and significant drop compared to other locations. Previous studies also showed significantly lower push out bond strength of MTA based sealer [57] and other types of root canal sealers [58] in the apical third of the roots compared to other locations. On the other hand, the present study showed significantly higher bond strength of IRM at the apical part of the roots compared to other locations. No previous studies have compared the bond strength of IRM in different location of the roots. The previously reported significant difference in dentinal tubule density [59, 60], orientation [59], and degree of tubular sclerosis [61] between apical and coronal part of the roots may be attributed to the observed significant change in the bond strength at the apical part of the roots.

Conclusion

The suggested prototype S-PRG root repair cement offered a significantly superior antibacterial effect against *P. gingivalis* bacterium compared to both MTA and IRM and significantly shorter initial and final setting time in comparison to MTA. Furthermore, S-PRG cement showed the ability to continuously release multiple ions up to four weeks including boron, fluoride and strontium. Both MTA and IRM had significantly higher push out bond strength than S-PRG in the coronal and middle third of the roots. Therefore, S-PRG cement may be used as a single-visit root repair material that can release multiple ions with potential

antibacterial effects against endodontic pathogens. Further studies are warranted to determine the biocompatibility of S-PRG cement.

Compliance with Ethical Standards

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human teeth were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figure 1A. The means (SE) of initial setting times of the three tested cements. Different letters represent statistically significant differences.

Figure 1B. The means (SE) of final setting times of the three tested cements. Different letters represent statistically significant differences.

Table 1. The mean percentage (SE) of bacterial growth after exposure to one- and three-day old root cements.

Type of cement	*E. faecalis*		*P. gingivalis*	
	1 day	**3 days**	**1 day**	**3 days**
S-PRG	91 (5.3)Ca	90 (4.2)Ca	5 (3)Da	1 (0.95)Ca
MTA	85(4.4)Da	86 (2.3)Da	105 (5.5)Aa	98 (6.9)Ab
IRM	98 (2.9)Ba	96 (2.1)Ba	89 (4.7)Ca	77(5.2)Bb
Control	100Aa	100Aa	100Ba	100Aa

Within each bacterium, different uppercase letters indicate a significant difference between different types of cements and the control (set at 100%) at each time point. Within each bacterium, different lowercase letters indicate a significant difference between one- and three-day old samples of the same cement.

Table 2

Table 2. Non-cumulative and cumulative release of ions from tested cements in distilled water.

Material	4 hours	1 day	3 days	1 week	2 weeks	4 weeks
Fluoride (ppm) released						
S-PRG (non-cumulative)	16 (4)D	41 (2)A	46(4)A	41 (3)A	34 (2)B	28 (1)C
S-PRG (cumulative)	16 (4)F	57 (3)E	103 (6)D	144 (4)C	178 (3)B	206 (3)A
MTA	0	0	0	0	0	0
IRM	0	0	0	0	0	0
Calcium (ppm) released						
S-PRG	0	0	0	0	0	0
MTA (non-cumulative)	43 (9)A	20 (1)B	21 (0.5)B	21 (2)B	24 (2)B	23 (2)B
MTA (cumulative)	44 (9)F	64 (9)E	85 (8)D	106 (11)C	130 (12)B	153 (12)A
IRM	0	0	0	0	0	0
Strontium (ppm) released						
S-PRG (non-cumulative)	7 (0.4)Ea	8 (1)Ea	27 (3)Da	60 (13)Ca	84 (5)Ba	97(6)Aa
MTA (non-cumulative)	9 (1)Ab	8 (1)Aa	3 (0.1)Bb	3(0.2)Bb	2 (0.3)Bb	2 (0.2)Bb
IRM (non-cumulative)	0.2 (0.02)Ac	0.2(0.02)Ab	0.2 (0.03)Ac	0.2 (0.03)Ac	0.2 (0.02)Ac	0.2 (0.006)Ac
S-PRG (cumulative)	7 (0.4)Fa	15 (2)Ea	42 (2)Da	102(13)Ca	186 (17)Ba	283 (23)Aa
MTA (cumulative)	9 (1)Fa	17 (0.5)Ea	20 (1)Db	22 (1)Cb	25 (0.3)Bb	27 (0.2)Ab
IRM (cumulative)	0.2 (0.02)Fb	0.4 (0.01)Eb	0.6 (0.03)Dc	0.8 (0.04)Cc	1 (0.05)Bc	1.2 (0.05)Ac
Boron (ppm) released						
S-PRG (non-cumulative)	481 (50)A	235 (22)B	168 (30)C	117 (18)D	80 (9)E	51 (4)F
S-PRG (cumulative)	481 (50)D	716 (43)C	884 (66)B	1002 (56)A	1082 (65)A	1133 (62)A
MTA	0	0	0	0	0	0
IRM	0	0	0	0	0	0
Aluminum (ppm) released						
S-PRG (non-cumulative)	2.7 (0.6)Ea	12 (0.8)Da	23 (4)Aa	18 (2)Ba	15 (0.5)Ca	12 (2.6)Da
IRM (non-cumulative)	0.07 (0.01)Ab	0.07 (0.01)Ab	0.06 (0.02)Ab	0.1 (0.005)Ab	0.09 (0.01)Ab	0.08 (0.01)Ab
S-PRG (cumulative)	2.7 (0.6)Fa	14.5 (1.1)Ea	37 (2.6)Da	55.1 (0.5)Ca	70.4 (1)Ba	82 (2.9)aA
IRM (cumulative)	0.07 (0.01)Fb	0.14 (0.02)Eb	0.2 (0.02)Db	0.28 (0.01)Cb	0.37 (0.004)Bb	0.5 (0.01)Ab
MTA	0	0	0	0	0	0
Zinc (ppm) released						
S-PRG (non-cumulative)	26 (7)Aa	15 (4)Ba	5 (0.2)Db	7 (2)Ca	10 (0.8)Ba	12 (1)Ba
IRM (non-cumulative)	0.7 (0.1)Cb	5 (2)Bb	11 (0.6)Aa	11 (1)Aa	11 (1)Aa	10 (0.4)Aa
S-PRG (cumulative)	26 (7)Da	41 (11)Ca	46 (11)Ca	53 (12)Ca	63 (13)Ba	75 (14)Aa
IRM (cumulative)	0.7 (0.1)Fb	5 (2)Eb	16 (2)Db	27 (3)Cb	38 (2)Bb	48 (2)Ab
MTA	0	0	0	0	0	0
Sodium (ppm) released						
S-PRG (non-cumulative)	957 (140)Aa	805 (69)Ba	962 (174)Aa	693 (90)Ba	522 (67)Ca	370 (12)Da
MTA (non-cumulative)	77 (5)Ab	90 (20)Ab	62 (11)Ab	41 (8)Ab	36 (1)Ab	44 (1)Ab
S-PRG (cumulative)	957 (140)Fa	1762(102)Ea	2723 (256)Da	3417 (188)Ca	3939 (250)Ba	4309 (262)Aa
MTA (cumulative)	77 (5)Fb	167 (16)Eb	229 (27)Db	270 (20)Cb	306 (21)Bb	350 (20)Ab
IRM	0	0	0	0	0	0
pH of distilled water						
S-PRG	7.5 (0.03)Ab	7.4 (0.09)ABb	7.4 (0.09)ABb	7.3 (0.08)ABb	7.1 (0.03)ABb	6.9 (0.04)Bb
MTA	7.9 (0.06)Aa	7.9 (0.04)Aa	8.1 (0.06)Aa	7.8 (0.2)ABa	7.6 (0.04)ABa	7.5 (0.07)BCa
IRM	7.3(0.2)Ac	7.2 (0.09)Ab	7.1 (0.3)ABb	6.6 (0.4)Bc	6.6 (0.5)Bb	6.2 (0.2)Cc
Water (negative control)	7.2 (0.3)Abc	7.2 (0.2)Ab	7.2 (0.2)Ab	7.1 (0.2)Ab	6.9 (0.2)Ab	6.7(0.1)Bb

Within each outcome, different lower case letters represent statistically significant differences between different materials and different upper case letters represent statistically significant differences between different time points for the same material. No measurable ions were detected from the negative control (distilled water) except traces of Na (15-25 ppm).

Table 3. Mean (SE) of push-out bond strength (MPa) of various tested root canal cements in the apical, coronal and middle part of the roots.

Root level	Type of root repair cement		
	IRM (MPa)	**MTA (MPa)**	**S-PRG (MPa)**
Apical	10.06 (2.63)Aa	1.71 (0.83)Bb	2.72 (1.55)Ab
Middle	8.25 (1.39)Bb	12.69 (3.03)Aa	4.38 (2.60)Ac
Coronal	7.82 (2.22)Bb	12.31 (2.68)Aa	4.50 (1.90)Ac

Within each cement type, different upper case letters indicate statistically significant differences. Within each location, different lower case letters indicate statistically significant differences.

Figure 1A

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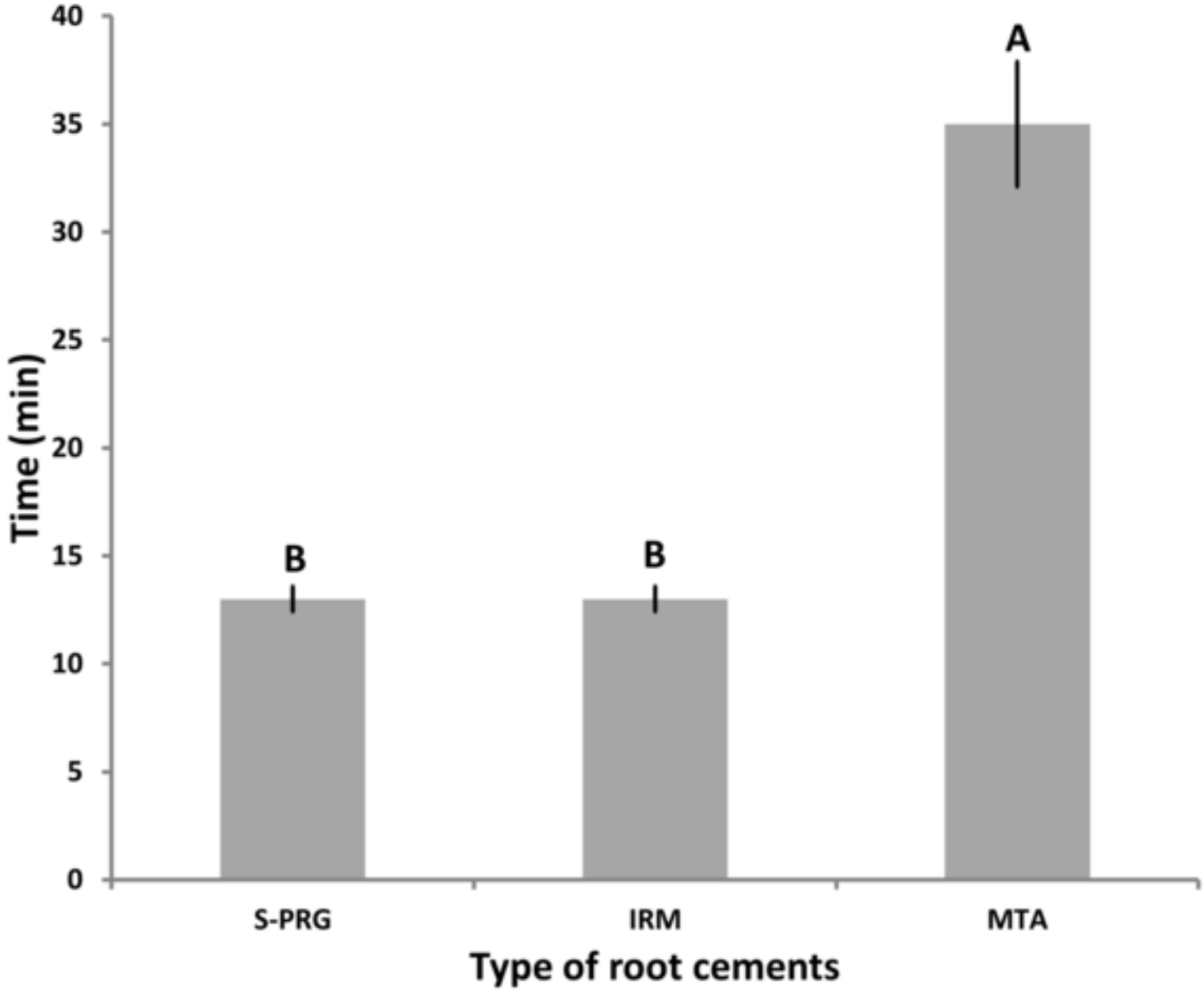

Figure 1B

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