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Effect of titanium tetrafluoride addition on the physicochemical and antibacterial properties of Biodentine as intraorfice barrier

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ABSTRACT

Objective. To evaluate the influence of titanium tetrafluoride (TiF_4) incorporation on the physicochemical and antibacterial properties of Biodentine (BD; Septodont, Saint Maur des Faussés, France) as an intraorfice barrier material.

Methods. Three different proportions of TiF_4 powder were used with BD; 1 wt%, 2 wt%, and 3 wt%; respectively. BD without TiF_4 addition was used as the control group. The setting time (ST) was determined using Gillmore needle apparatus. Diametral tensile strength (DTS) and fracture resistance were measured in a universal testing machine. Solubility was assessed using mass variation after 7 days water storage. The hardness test was conducted using Vickers microhardness tester. The antibacterial activity was assessed using direct contact test against *Enterococcus faecalis*. Radiopacity was assessed and expressed in thickness of aluminum. Surface topography and elemental composition of modified BD were also assessed. The pH of soaking water was measured up to 168 h. Data of tested properties were analyzed using one-way analysis of variance, the paired *t*-test, two-way repeated measures analysis of variance, and Tukey post hoc tests ($P < 0.05$).

Results. BD-incorporating 2 wt% TiF_4 revealed the highest surface microhardness, DTS, and fracture resistance compared with the unmodified group ($P < 0.001$). Higher concentrations of TiF_4 (3 wt%) compromised the solubility and prolonged the ST of BD ($P < 0.05$). Bacterial growth of BD-incorporating TiF_4 was significantly reduced when compared with the control group ($P < 0.05$). The tested materials induced alkalization of the soaking water that decreased with time.

Significance. Biodentine-incorporating TiF_4 (1 wt% and 2 wt%) is a promising intraorfice barrier material with enhanced physicochemical and antibacterial properties.

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1. Introduction

Calcium silicate-based materials (CSMs) are widely used for various endodontic applications such as repair for perforation, pulp capping, apexification, intraorfice barriers, and root-end filling materials [1–3]. CSMs showed enhanced biological properties and the capability to produce an appropriate bioactive surface with the nucleation of calcium phosphates and the development of an apatite layer after immersion in different simulated body fluids [4–6]. Consequently, CSMs have achieved a substantial consideration for several clinical applications [4].

Biodentine (Septodont, Saint Maur des Faussés, France) has been developed as the second generation of CSMs to overcome the disadvantages of mineral trioxide aggregate (MTA) [7]. The main disadvantages of MTA are prolonged setting time, difficult handling characteristics, and potential of tooth discoloration [8]. Biodentine has been introduced to combine the high biocompatibility and bioactivity of CSMs, with improved properties such as quick setting time due to the calcium chloride added to the Biodentine liquid and enhanced mechanical properties as claimed by the manufacturer [9]. Various modifications have been performed to enhance the physical properties of Biodentine including glass-fiber, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and bioactive glass nanoparticles [10–13].

The concept of intraorfice barrier has been introduced to prevent the coronal microleakage [14]. In addition, it has been suggested that the intraorfice barrier could be utilized to provide resistance against forces that produce root fractures [15]. It has been reported that the reinforcing effect depends on the type of material utilized as intraorfice barrier [15]. Fiber-reinforced composite and resin-modified glass ionomer intraorfice barriers significantly enhanced the resistance against root fracture compared with MTA [15]. Recently, it has been suggested that incorporation of glass-fiber with ProRoot MTA (Dentsply Sirona, York, PA, USA) and Biodentine could enhance the reinforcing effect of intraorfice barriers in root-filled teeth [10]. Development of a new/modified reinforced intraorfice barrier material to enhance the fracture resistance, coronal seal, and antibacterial activity would be beneficial to patient and endodontists.

Titanium tetrafluoride (TiF_4) has been evaluated for different applications in dentistry including retarding the development of carious lesion [16], preventing dental hypersensitivity and erosion [17,18], sealing dentinal tubules of root canal dentine [17], and antibacterial agent [19]. It has been shown that the compressive strengths of 0.5 and 1% TiF_4-added glass-ionomer cement were higher compared with the unmodified; however it was not statistically significant [20]. Therefore, in the present study, higher concentrations of TiF_4 (1 wt%, 2 wt%, and 3 wt%) were added to Biodentine. Consequently, it could be postulated that the positive effect of TiF_4 might help to enhance the physicochemical and antibacterial properties of Biodentine as an intraorfice barrier material. Thus, the aim of this study was to evaluate the effect of TiF_4 addition on the physicochemical and antibacterial properties of Biodentine as intraorfice barrier material. The null hypothesis tested was that the addition of TiF_4 has no influence on the physicochemical and antibacterial properties of Biodentine.

Table 1 – Chemical composition of Biodentine.

Powder	Liquid
Tricalcium silicate ($3CaO{\cdot}SiO_2$) Dicalcium silicate ($2CaO{\cdot}SiO_2$) Calcium carbonate ($CaCO_3$) Zirconium dioxide (ZrO_2) Iron oxide	Calcium chloride ($CaCl_2{\cdot}2H_2O$) Hydrosoluble polymer

2. Materials and methods

Commercially available Biodentine (BD; Septodont) powder was mixed with different proportion of TiF_4 powder (Aldrich Chemical Company, Milwaukee, WI, USA). The chemical composition of BD is presented in Table 1 [21]. Three different proportions of TiF_4 powder were used with BD; 1 wt%, 2 wt%, and 3 wt%, respectively. BD without TiF_4 addition was used as the control group. The modified BD with added TiF_4 powders was mixed using an amalgamator (Silamat; Ivoclar Vivadent AG, Liechtenstein) before mixing with the BD liquid. For each specimen prepared in a mould; the following procedures were performed: each mould was placed on a glass slide covered with a plastic plate, and the mix was then compacted into the mould until it was filled. Then, another plastic plate and a glass slide were placed on the mould. A 100 g load was placed on the mould for 1 min and the excess material was wiped away with a wet cotton pellet. After that, each specimen inside the mould was placed in an incubator at 37 °C with 100% relative humidity for 24 h. Then, the specimen was gently removed from the split mould [22,23]. The damaged specimen during preparation was discarded and another acceptable specimen was prepared.

2.1. *Setting time*

Sixty rectangular-shaped specimens (n = 15) were prepared by placing the mixed cement inside a stainless-steel rectangular mould (10 mm × 8 mm × 5 mm). The setting time was determined using Gillmore needle apparatus with a flat-end cylindrical stainless steel tip (initial Gillmore tip; 113.4 g weight and 2.12 mm diameter and final Gillmore tip; 453.6 g weight and 1.06 mm diameter). The needle was lowered vertically on the surface of the specimen until the needle failed to leave a mark on the specimen surface [12,24–26].

2.2. *Diametral tensile strength*

Sixty cylindrical-shaped specimens (6 mm in diameter and 4 mm in height) (n = 15) were prepared by placing the mixed material in a stainless-steel split mould. The specimens inside the mould were placed in an incubator at 37 °C with 100% relative humidity for 24 h. Then, each specimen was immersed in 10 mL deionized distilled water for 24 h at 37 °C. After that, each specimen was tested using a universal testing machine (Lloyd LRX; Lloyd Instruments Ltd., Fareham, UK) at a crosshead speed of 1 mm/min. The diametral tensile strength

(MPa), was calculated according to the following formula [10]:

$$Diametral\ tensile\ strength = \frac{2P}{\pi Dt}$$

where P is the load in Newtons at fracture, D is the diameter of the specimen in mm and t is the thickness of specimen in mm.

2.3. Solubility

Sixty disc-shaped specimens (15 mm in diameter and 1 mm in thickness) (n = 15) were prepared by placing the mixed material in a stainless-steel split mould. The specimens were stored for 24 h at 37 °C. Then, the specimens were demoulded and weighed in order to record their mass (m_1). The thickness and diameter of each specimen were measured and the volume (V) was calculated in mm^3. Then, each specimen was immersed in 50 mL deionized distilled water for 7 days at 37 °C. After that, the specimens were removed, dried by blotting with absorbent paper and placed in a desiccator. The specimens were desiccated until a constant mass (m_2) was attained. The water solubility (W_{sl}) was calculated using the formula [24]:

$$W_{sl}(\%) = \frac{m_1 - m_2}{V} \times 100$$

2.4. Surface microhardness

Sixty disc-shaped specimens (6 mm in diameter and 3 mm in thickness) (n = 15) were prepared by placing the mixed material in a stainless-steel split mould. The surface microhardness was performed by using a Vickers microhardness tester (Mitutoyo, Mitutoyo Asia Pacific Ltd., Singapore) with a diamond shaped indenter. The load was 50 g for 10 s. Five indentations were made on the surface of each specimen and the mean Vickers hardness number (VHN) value was recorded [23,27].

2.5. Antibacterial activity

The antibacterial activity of different tested modified BD was determined using the direct contact test (DCT) [28]. Briefly, the sidewalls of eight wells in the 96-well microtiter plate (Nunc, Wiesbaden, Germany) were coated uniformly with each of the freshly mixed test materials (plate A). A 10 μL of bacterial suspension (3×10^8 colony-forming units (CFU)/mL, which contained 3×10^6 bacteria) of *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212) was placed on the surface of each material. Then, brain heart infusion broth (BHI) (235 μL) was added to plate A. A 15 μL were then transferred from the wells of plate A to another plate (plate B), into an adjacent set of 8 wells containing fresh medium (205 μL).

Positive control is the uncoated wells which exposed to bacteria. However, the negative control is the coated wells with the test materials with BHI and without bacteria. The antibacterial activity was evaluated 1 h after mixing and after aging in 280 μL of phosphate-buffered saline for 7 days at 37 °C. The bacterial outgrowth in each well was followed by continuous densitometric measurements at 620 nm every 30 min for 15 h using a microplate spectrophotometer (Tecan Freedom EVO, Männedorf, Switzerland) at 37 °C. The baseline values were obtained from the negative control wells which were then deducted from the respective experimental data. Data were recorded in optical density (OD) units.

2.6. Fracture resistance

Seventy-five extracted human mandibular premolars (n = 15) single rooted with single canal and less than 10° curvature with approximately same dimension were used and examined by a stereomicroscope (SZR-10; Optika, Bergamo, Italy) to ensure the absence of preexisting cracks. The teeth were reduced to a standardized root length of 14 mm as measured from the coronal aspect [10,29].

The root canals were instrumented using Mtwo nickel-titanium instruments (VDW, Munich, Germany) up to size 25, .06 taper. The canals were irrigated with 5.25% NaOCl throughout instrumentation, followed by 17% EDTA final irrigation. A 10 mL distilled water was utilized to flush the canals to avoid the extended effect of EDTA and NaOCl [15]. The canals were obturated using a gutta-percha (single cone size 25, .06 taper) and a resin-based endodontic sealer (AH Plus; Dentsply De Trey, Konstanz, Germany). After that, the specimens were stored for 2 weeks at 37 °C and 100% humidity to allow complete set of the sealer. Fifteen specimens were randomly chosen as the negative control group (without intraorfice barrier). The coronal 3 mm of root canal obturation was removed using a heated spoon excavator and later on with 70% alcohol-moistened microbrushes to remove sealer remnants. Then, the specimens were randomly divided into 4 groups (n = 15) according to the modified BD intraorfice barrier used over root canal obturation. The specimens contained BD without modification is the positive control group. After that, the specimens were stored at 37 °C and 100% humidity for 3 weeks [10,29].

Simulation of periodontal membrane was performed as described previously [30–32]. Then, the specimens were mounted on a universal testing machine (Lloyd LRX) and a compressive force was applied to each specimen at a crosshead speed of 1 mm/min until vertical root fracture occurred [10,29]. The peak load to fracture was recorded in newtons (N). Fractured specimens were sputtered with gold and examined using a scanning electron microscopy (SEM) (Zeiss LEO 440, LEO Electron Microscopy LTD, Cambridge, UK).

2.7. Radiopacity

Sixty-shaped specimens (10 mm diameter × 1 mm height) (n = 15) were prepared and radiographed according to the ISO 6876 [33]. The set specimens were radiographed using a dental X-ray unit (Anthos, Imola, Italy) with an exposure time of 0.80 s at 10 mA and 70 kV with a 300 ± 10 mm distance. A reference aluminum step wedge of 60 mm length and 10 mm width was used. The radiographs were processed and scanned. The radiographic density data were transformed into aluminum step wedge equivalent thickness (mm Al) using ImageJ software (http://rsbweb.nih.gov/ij; National Institutes of Health, Bethesda, MD) [24,26,34].

2.8. Surface topography and elemental composition

Twenty disc-shaped specimens (n = 5) were prepared in a stainless-steel split mould (10 mm in diameter and 2 mm in

thickness) for surface topography evaluation using a SEM. The specimens were sputtered with gold, mounted on aluminum stubs and then examined. The surface of each specimen was examined for detecting surface modifications including deposits and porosity. Specimens were analyzed for elemental composition using energy-dispersive X-ray spectroscopy (EDX) (Isis System Series 200; Link Analytical, Lidingö, Sweden) at × 500 magnification with accelerating voltage of 20 kV using secondary electron imaging.

2.9. *pH assessment*

Sixty disc-shaped specimens (n = 15) were prepared in a stainless-steel split mould (8 mm in diameter and 1.6 mm in thickness) for pH evaluation. Then, each specimen was immersed in 10 mL of deionized water and stored at 37 °C. The soaking water was collected and replaced at four endpoints (3, 24 h, 72 h, and 168 h) [26]. The collected water was analyzed for pH using a pH meter (pH/mV/Temp Meter Set, SP-2100; Suntex, Taipei, Taiwan).

2.10. *Statistical analysis*

The data of setting time, diametral tensile strength, solubility, microhardness, fracture resistance, antibacterial activity, and radiopacity were statistically analyzed (SPSS 23.0; IBM Software, Armonk, NY, USA) using a one-way analysis of variance. For the antibacterial activity, the paired *t*-test was performed to compare between the two different time intervals within groups. The data of pH were analyzed by two-way repeated measures analysis of variance. Multiple comparisons were performed using Tukey post hoc tests. Statistical significance level was set at $P < 0.05$.

3. Results

The data of setting time, diametral tensile strength, solubility, microhardness, radiopacity, and pH are presented in Table 2. BD + TiF_4 (3 wt%) revealed the highest initial setting time among the groups ($P < 0.001$). There was a statistically significant difference in the initial setting time between the groups ($P < 0.001$). There was no significant difference in the final setting times between BD + TiF_4 (3 wt%) and BD + TiF_4 (2 wt%) ($P = 0.408$). Also, there was no significant difference in the final setting times between BD + TiF_4 (1 wt%) and BD + TiF_4 (2 wt%) ($P = 0.408$). The unmodified BD exhibited the shortest initial and final setting times.

The results of diametral tensile strength and microhardness for the tested materials revealed that there were significant differences among the groups ($P < 0.001$). BD + TiF_4 (2 wt%) exhibited the highest diametral tensile strength and microhardness values ($P < 0.001$). It was observed that BD + TiF_4 (3 wt%) revealed the lowest diametral tensile strength and microhardness among the groups ($P < 0.001$). There was no statistically significant difference between the control, BD + TiF_4 (1 wt%), and BD + TiF_4 (2 wt%) in the solubility ($P > 0.05$). However, BD + TiF_4 (3 wt%) revealed higher solubility value compared with the other groups ($P < 0.05$).

Table 2 – Mean (Standard deviations) of setting time, diametral tensile strength, solubility, hardness, radiopacity, and pH of modified Biodentine with statistical analysis.

Groups	Setting time (min)		Diametral tensile strength (MPa)	Solubility (%)	Microhardness (VHN)	Radiopacity (mm Al)	pH*			
	Initial	Final					3 h	24 h	72 h	168 h
Control	14 (1.1)^{A}	44 (2.6)^{A}	15.3 (0.4)^{B}	4.9 (0.4)^{A}	49.6 (2.1)^{B}	4.2 (0.4)^{A}	11.4 (0.5)^{Ba}	11.6 (0.4)^{Ba}	10.5 (0.8)^{Bb}	9.6 (0.6)^{Bc}
BD + TiF_4 (1 wt%)	16 (1.1)^{B}	48 (2.9)^{B}	20.1 (1.1)^{C}	5.0 (0.5)^{A}	55.4 (2.1)^{C}	4.5 (0.3)^{AB}	11.1 (0.7)^{ABa}	11.3 (0.6)^{ABa}	10.1 (0.8)^{ABb}	9.1 (0.5)^{ABc}
BD + TiF_4 (2 wt%)	18 (1.7)^{C}	50 (4.2)^{BC}	24.3 (0.9)^{D}	5.1 (0.3)^{A}	60.7 (2.3)^{D}	4.7 (0.3)^{BC}	10.9 (0.8)^{ABa}	11.1 (0.8)^{ABa}	9.8 (0.6)^{ABb}	8.9 (0.7)^{ABc}
BD + TiF_4 (3 wt%)	23 (1.3)^{D}	52 (3.8)^{C}	14.1 (0.4)^{A}	5.5 (0.3)^{B}	41.8 (2.2)^{A}	4.9 (0.3)^{C}	10.5 (0.9)^{Aa}	10.9 (0.6)^{Aa}	9.4 (0.7)^{Ab}	8.4 (0.8)^{Ac}

Mean values for each property represented with different superscript uppercase letters (column) are significantly different ($P < 0.05$).

* Mean values represented with different superscript lowercase letters (row) are significantly different ($P < 0.05$).

Table 3 – Mean (standard deviations) of antibacterial activity and fracture resistance ofmodified Biodentine with statistical analysis.

Groups	Antibacterial activity*		Fracture resistance (N)
	1 h	7 days	
Negative control	0.81 (0.02)^{Ca}	4.67 (0.35)^{Bb}	641.2 (41.6)^{A}
Positive control	0.87 (0.03)^{Ca}	4.39 (0.21)^{Bb}	828.3 (55.1)^{C}
BD + TiF_4 (1 wt%)	0.55 (0.03)^{Ba}	1.40 (0.11)^{Ab}	881.7 (50.4)^{D}
BD + TiF_4 (2 wt%)	0.48 (0.03)^{Aa}	1.31 (0.08)^{Ab}	950.7 (42.3)^{E}
BD + TiF_4 (3 wt%)	0.46 (0.04)^{Aa}	1.30 (0.09)^{Ab}	731.1 (48.5)^{B}

Mean values represented with different superscript uppercase letter (column) are significantly different ($P < 0.05$).
* Mean values for antibacterial activity represented with different superscript lowercase letter (row) are significantly different ($P < 0.05$).

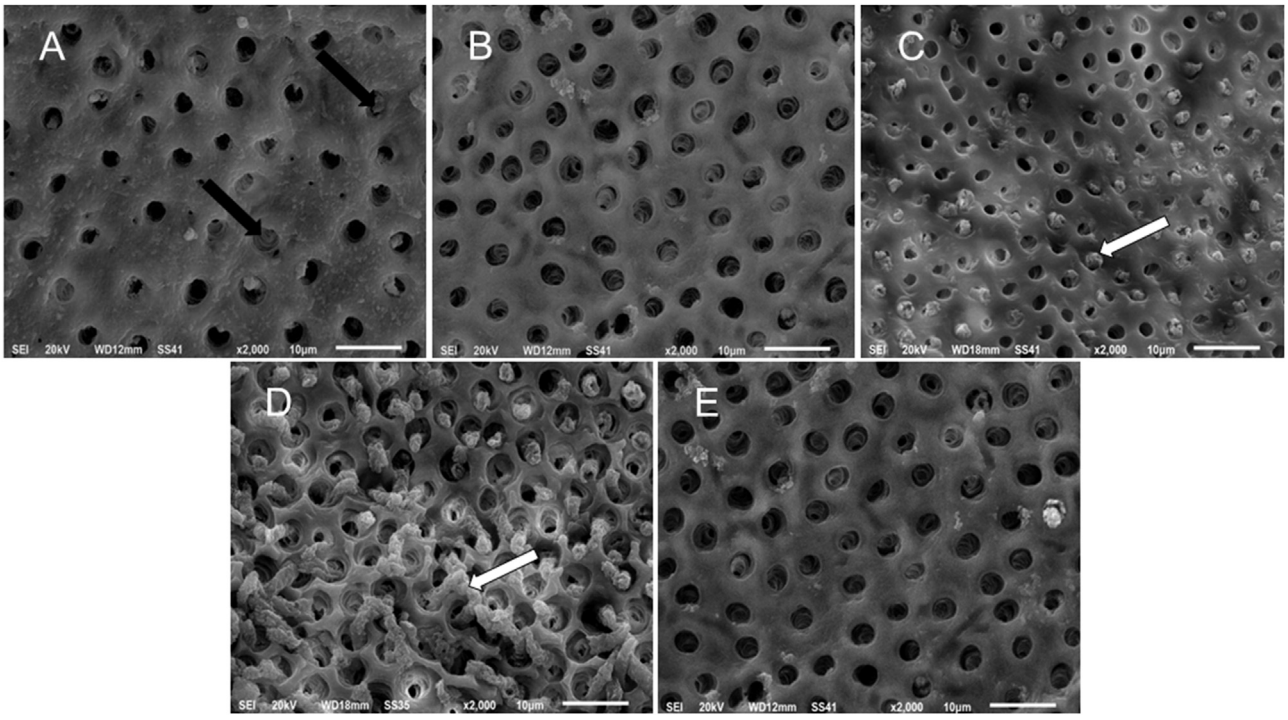

Fig. 1 – Representative SEM images (×2000) of fractured specimens from fracture resistance test of different groups. A; negative control (without intraorfice barrier) showing that the dentinal tubules are partially filled with the resin sealer (black arrow), B; positive control (Unmodified BD), C; BD + TiF_4 (1 wt%), D; BD + TiF_4 (2 wt%) and E; BD + TiF_4 (3 wt%). White arrow represents remnants of BD in the dentinal tubules with presence of tags (C, D).

Regarding the radiopacity, all the tested groups revealed a radiopacity value higher than 3 mm Al. A radiopacity equal or higher than 3 mm Al is required by the ISO 6876 [33]. BD + TiF_4 (3 wt%) was the most radiopaque material. There was no significant difference between BD + TiF_4 (3 wt%) and BD + TiF_4 (2 wt%) in radiopacity ($P = 0.336$). However, BD + TiF_4 (3 wt%) and BD + TiF_4 (2 wt%) revealed higher radiopacity than the control group ($P = 0.001$ and $P = 0.007$; respectively).

The tested materials induced alkalization of the soaking water that decreased with time (Table 2). The pH decreased starting at 72 h continuing till 168 h. No significant differences were detected between the control, BD + TiF_4 (1 wt%), and BD + TiF_4 (2 wt%) at all tested times ($P > 0.05$). However, BD + TiF_4 (3 wt%) revealed a more rapid decrease in alkalization compared with the control group at all tested times ($P < 0.05$). There was no significant difference between BD + TiF_4 (1 wt%), BD + TiF_4 (2 wt%), and BD + TiF_4 (3 wt%) at all tested times ($P > 0.05$). At 72 h and 168 h, the pH of each material was significantly decreased compared with the 3 h and 24 h ($P > 0.05$).

The data of antibacterial activity and fracture resistance are presented in Table 3. The antibacterial effects of modified BD set for 1 h and 7 days revealed an inhibitory effect on the growth of *E. faecalis* ($P < 0.001$). Bacterial growth of BD incorporating TiF_4 was significantly reduced when compared with the control group ($P < 0.001$). After 7 days, the modified BD maintained their antibacterial activity ($P < 0.001$). Fracture resistance of roots with BD + TiF_4 (2 wt%) intraorfice barrier revealed the highest reinforcing effect among the tested groups ($P < 0.001$). There was a significant difference in the fracture resistance among the tested groups ($P < 0.001$). The ranking of fracture resistance for the tested groups

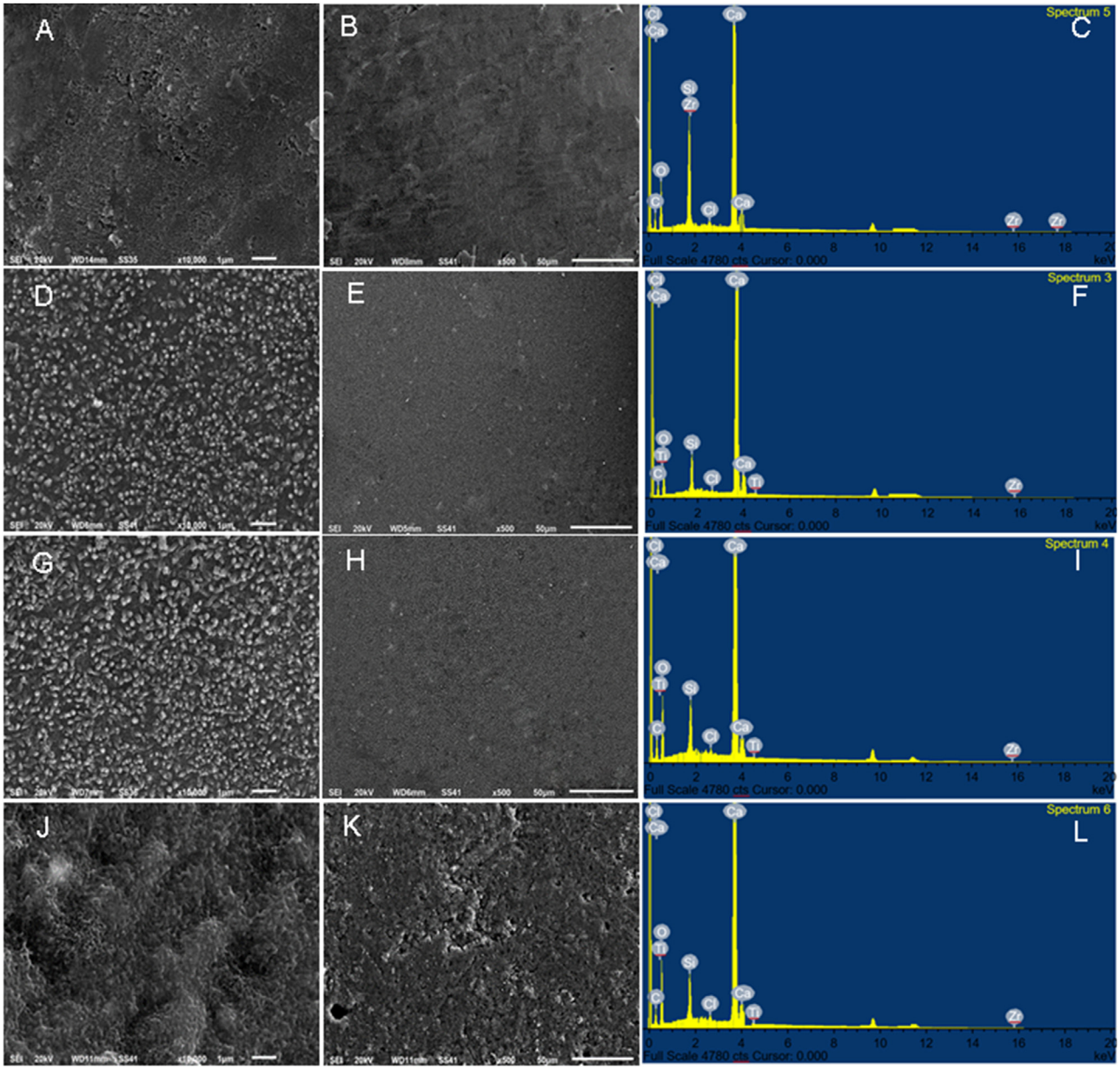

Fig. 2 – SEM images (×10,000) and EDX analysis of BD surface: A; Unmodified BD, D; BD + TiF_4 (1 wt%), G; BD + TiF_4 (2 wt%) and J; BD + TiF_4 (3 wt%). Representative SEM images (× 500) (B, E, H, K) with EDX elemental analysis (C, F, I, L).

was as follow: BD + TiF_4 (2 wt%) > BD + TiF_4 (1 wt%) > positive control > BD + TiF_4 (3 wt%) > negative control (Table 3). Representative SEM images of fractured specimens are presented in Fig. 1. The negative control group showed that the dentinal tubules were partially filled with the resin sealer (Fig. 1A). The positive control showed some dentinal tubules were filled with BD (Fig. 1B). BD + TiF_4 (2 wt%) showed more tags of BD in the root dentinal tubules (Fig. 1D) compared with BD + TiF_4 (1 wt%) and BD + TiF_4 (3 wt%) (Fig. 1C and E; respectively).

SEM analysis of the topography surface of BD in the control group revealed that the surface was homogenous with small black areas interpreted as pores (Fig. 2A). The EDX analysis revealed that the elemental composition of BD is composed of calcium (Ca), silicon (Si), carbon (C), oxygen (O), zirconium (Zr), and chlorine (Cl) (Table 4, Fig. 2C). BD modified by TiF_4 revealed small granular particles (Fig. 2D and G). These granules composed mainly of titanium element as analyzed by EDX (Fig. 2F, I, and L). With increasing the amount of incorporated TiF_4 (3 wt%), it was observed larger globular precipitates

Table 4 – EDX analysis (at%) of the Biodentine surfaces of different groups.

Groups	Elemental composition (at%)						
	Ca	Si	O	C	Cl	Zr	Ti
Control	29.5	14.8	27.6	18.3	9.1	0.7	–
BD + TiF_4 (1 wt%)	25.3	11.5	24.6	15.3	8.2	0.5	14.6
BD + TiF_4 (2 wt%)	23.3	10.2	22.1	11.3	7.5	0.4	25.2
BD + TiF_4 (3 wt%)	20.2	9.6	21.3	10.5	6.4	0.2	31.8

formed on the surface with black areas interpreted as pores (Fig. 2J and K). Representative SEM/EDX images for each group are presented in Fig. 2.

4. Discussion

The present study evaluated the addition of TiF_4 to BD CSMs with different amount (1 wt%, 2 wt%, and 3 wt%) on the physicochemical and antibacterial properties of BD as intraorfice barrier material. The null hypothesis was rejected because there were differences in the physicochemical and antibacterial properties between BD-containing 1 wt%, 2 wt%, 3 wt% TiF_4, and unmodified BD.

The unmodified BD revealed lower setting time than the other groups. The presence of calcium chloride in the mixing liquid and calcium carbonate in the BD powder reduced the setting time as compared with other CSMs materials in previous studies [12,24,35]. In the present study, the final setting time of unmodified BD was approximately 44 min which is in agreement with a previous study [24]. It was observed that by increasing the amount of TiF_4 incorporation in the powder of BD, both the initial and final setting times were increased. This finding could be attributed to the addition of TiF_4 that might prolong the setting times of modified BD. It could be postulated that the addition of TiF_4 might interfere with the setting reaction of BD and accordingly delaying the matrix formation and increasing the setting time. It has been reported that the addition of any substance interfere with the setting reaction of CSMs-based materials [12,36]. However, the setting times for BD incorporated 1 wt% and 2 wt% TiF_4 still acceptable as the differences between these modified groups and unmodified BD ranged from 2–6 min. On the other hand, BD-containing 3 wt% TiF_4 revealed longer setting times compared with the unmodified BD.

It was observed that the addition of 1 wt% and 2 wt% TiF_4 to BD improved the diametral tensile strength, microhardness, and fracture resistance properties compared with the unmodified BD. However, these properties were decreased by increasing the amount of TiF_4 incorporation (3 wt%). BD + TiF_4 (3 wt%) revealed the lowest diametral tensile strength, microhardness, and fracture resistance among the groups. Improvements in the fracture resistance of BD incorporated 1 wt% and 2 wt% TiF_4 were supported by surface topography of fractured specimens. There were more tags resulted from BD penetration in the dentinal tubules of root dentin compared with the other groups. Enhancement of the mechanical properties of modified BD with 1 wt% and 2 wt% TiF_4 as intraorfice barriers might indicate that the addition of TiF_4 with adequate amount improved the reinforcement of root-filled teeth. The concept of intraorfice barriers was initially performed to prevent the coronal microleakage [14]. After that, it was also indicated to be used to reinforce root-filled teeth against vertical root fracture [10,15].

High solubility of CSMs is undesirable because it will affect the sealing ability of CSMs and consequently facilitating the entrance of bacteria and microleakage [37,38]. There was no significant change in the solubility (%) value between the control, BD + TiF_4 (1 wt%) and BD + TiF_4 (2 wt%). On the other hand, BD + TiF_4 (3 wt%) revealed higher solubility value compared with the other groups. It could be postulated that the higher amount of TiF_4 (3 wt%) incorporation impeding the setting reaction and the hydration process and consequently less denser microstructures that might increase the solubility of BD. It should be emphasized that the *in vitro* solubility test of CSMs in distilled water does not predict the real stability and integrity of these materials under *in vivo* situations [26]. CSMs form soluble calcium salts and calcium hydroxide during the hydration and setting reactions which are rapidly washed out by water and resulted in pores formation [39]. However, when CSMs immersed in body fluid, the calcium phosphate deposits are formed that could enhance the sealing ability at the dentine-cement interface and fill the open voids [40]. It could be postulated that the results of the present study provide an initial indication about the solubility behaviour of modified BD with TiF_4.

The radiopacity evaluation revealed that all tested materials had a higher radiopaque value than 3 mm Al, as recommended by the ISO 6876 [33]. The zirconium oxide is one of the components of BD material and it acts as a radiopacifier [21]. It was observed that both BD + TiF_4 (3 wt%) and BD + TiF_4 (2 wt%) groups presented with higher radiopacity compared with the control group. The higher radiopacity value of modified BD could be contributed to the presence of titanium element beside zirconium oxide which is in the component of unmodified BD. EDX analysis showed the existence of titanium element on the surface of modified BD. It was observed that there was no proportionality in the amount of titanium detected on the surface of tested specimens with increasing the amount of TiF_4 addition to BD as shown in Table 4. This finding could be explained as the TiF_4 addition was not homogenously distributed within the whole structure of the material.

The reaction products resulted from mixing BD powder with the liquid consists of a hydrated calcium silicate gel and calcium hydroxide. The calcium hydroxide produced from the liquid phase [24,35,41,42]. In aqueous solution, TiF_4 dissociates and reacts into $TiOF_2$ and 2HF [43]. TiF_4 hydrolysis can result in the formation of many fluoride complexes [44]. It has been reported that fluoride ion has a great affinity to bind with silicon and calcium rather than to oxygen [43,45]. In addition, it could be speculated that fluoride element was not detected because of its low atomic weight [46,47]. Similarly, the fluoride element was not found on the surface of enamel that was treated by 4% TiF_4 varnish [45].

It was observed that the surface topography of BD showed small black areas interpreted as pores. The apparent porosity could be attributed to the higher reactivity and the capacity to release ions. The hydration reaction of calcium silicate particles results in dissolution of their surface with the formation of a calcium silicate hydrate gel and calcium hydroxide, together with the release of calcium and hydroxyl ions [4,24,41,42]. BD-containing TiF_4 showed small granules particles composed mainly of titanium element. Higher concentration of TiF_4 incorporation showed larger globular precipitates formed on the surface.

The use of an intraorfice barrier material with antibacterial activity would enhance the outcomes of endodontic treatment. The CSMs possess an alkalinizing activity which correlated with calcium hydroxide release that enhances the

antibacterial properties of these materials [4,48]. Experimental BD incorporating TiF_4 displayed bacteriostatic effect, representing inhibition of bacterial growth compared with the control group, both fresh (1 h) and aged (7 days). This finding could be attributed to the presence of fluoride and titanium elements. It had been reported that TiF_4 had an antibacterial activity against *Streptococcus Mutans* [49]. The high pH provides an antibacterial activity against common endodontic pathogens. It has been reported that pH 10.5–11.0 retards growth of *E. faecalis*, whereas no growth occurs at pH 11.5 [48]. In the present study, the alkalinizing activity of the tested materials decreased with the material aging. The amount of TiF_4 incorporation (1 wt% and 2 wt%) did not affect significantly the alkalinizing activity of BD compared with the control group. On the other hand, BD + TiF_4 (3 wt%) showed a more rapid decrease in the alkalinizing activity compared with the control group at all tested times. The pH values of BD + TiF_4 (1 wt%), BD + TiF_4 (2 wt%), and BD + TiF_4 (3 wt%) were not significantly different at all tested times. This finding could be attributed to the lower concentrations of TiF_4 incorporation that did not impair the alkalinizing activity of the modified BD material.

This study has highlighted the possible potential consideration of BD incorporating TiF_4 (1 wt% and 2 wt%) as an intraorfice barrier material without compromising the physicochemical and antibacterial properties of the material. Further studies are required to understand the formed microstructure. Additionally, due to the limitations of *in vitro* studies, clinical investigations are required to provide more relevant clinical data about BD incorporating TiF_4 as an intraorfice barrier material.

5. Conclusions

1. BD-incorporating 1 wt% and 2 wt% TiF_4 improved the DTS, surface microhardness and fracture resistance compared to the unmodified.
2. BD-incorporating 3 wt% TiF_4 compromised the mechanical properties, prolonged the ST and increased the solubility.
3. The addition of TiF_4 enhanced the antibacterial activity of BD against *E Faecalis*.
4. BD-incorporating 1 wt% and 2 wt% TiF_4 is a promising intraorfice barrier material.

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