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The effect of adding ytterbium trifluoride on the radiopacity, compressive strength, setting time and bioactivity of biodentine: an *in vitro* study

Efeito da adição de trifluoreto de itérbio na radiopacidade, resistência à compressão, tempo de presa e bioatividade da biodentina: estudo *in vitro*

Nareman Mahmoud BAHAA[1], Dalia Ibrahim EL-KORASHY[2], Dalia Ibrahim SHERIEF[3]

1 - Egyptian Russian University, Faculty of Dentistry, Biomaterials Department. Cairo, Egypt.
2 - Ain-shams University, Head of Dental Biomaterials Department, Faculty of Dentistry. Al Waili, Cairo, Egypt.
3 - Ain-shams University, Faculty of Dentistry, Dental Biomaterials department, Cairo, Egypt.

ABSTRACT

Objective: This study aimed to evaluate the radiopacity, compressive strength, setting time and bioactivity of Biodentine after modification with Ytterbium Tri-Fluoride (YbF_3) in three different concentrations. **Material and Methods:** Radiopacity was determined using the equivalence in millimeters of aluminum (mm Al) from digital radiographs. Compressive strength was evaluated using a universal testing machine. The initial and final setting times were evaluated using Gillmore needle. The bioactive potential was evaluated using the environmental scanning electron microscope (ESEM) connected with Energy Dispersive X-ray analysis (EDX) and X-ray Diffractometer (XRD) at three different time intervals. pH was measured using a pH-meter. Data were analyzed using one-way ANOVA followed by Tukey's post hoc tests ($P \leq 0.05$). **Results:** Radiopacity of Biodentine with 2.5%, 5% and 7.5% YbF_3 was significantly higher than unmodified Biodentine ($P \leq 0.05$). Unmodified Biodentine showed the highest mean compressive strength values compared to all other groups ($p \leq 0.05$). The addition of YbF_3 to Biodentine has extended the final setting time except for the 2.5% YbF_3 group that showed no significant difference compared to the control. All groups showed an alkaline pH at 28 days, ESEM coupled with EDX analysis showed evidence of dense globules of calcium phosphate on the surface indicating enhancement of bioactivity. **Conclusion:** 2.5% YbF_3 can be a promising radiopacifying agent to Biodentine with improvement in radiopacity, bioactive potential and maintaining the setting time and compressive strength at acceptable level as indicated by the ISO standards.

KEYWORDS

Ytterbium trifluoride; Radiopacity; Biodentine; Bioactivity.

RESUMO

Objetivo: O estudo buscou avaliar a radiopacidade, resistência à compressão, tempo de presa e bioatividade da Biodentina modificada por Trifluoreto de itérbio (YbF_3). **Materiais e Métodos:** YbF_3 foi incorporado ao pó de Biodentina e dividido em 4 grupos (X0, X1, X2 e X3) de acordo com a concentração de YbF_3; 0%, 2.5%, 5% e 7.5% do peso, respectivamente. Os espécimes foram preparados para cada experimento com um total de 40 espécimes para cada teste (n=10 por grupo). A radiopacidade foi determinada através do uso equivalente em milímetros de alumínio. A resistência a compressão foi avaliada através do uso de uma máquina de teste universal. O tempo inicial e final foi avaliado através de uma agulha de Gillmore. O potencial bioativo foi avaliado através do Microscópio Eletrônico de Varredura (ESEM), conectado com a análise de espectroscopia de raios X por energia dispersiva (EDX) e difração de raio X (DRX). O pH foi mensurado através do uso de um pHmetro. Os dados foram analisados pelo ANOVA seguido do Test Tukey. **Resultados**: A radiopacidade nos grupos X1, X2 e X3 foi significantemente maior do que no grupo X0. O grupo X0 mostrou a maior resistência à compressão comparado com os outros grupos. A adição de YbF_3 a Biodentina excedeu o tempo final exceto pelo grupo X1, que não

apresentou diferença estatística significante quando comparado com o grupo X0. Todos os grupos apresentaram um pH alcalino com 28 dias. ESEM com análise EDX mostrou evidência de densos glóbulos de fosfato de cálcio na superfície, indicando uma melhora na bioatividade. **Conclusão:** 2.5% YbF_3 é um promissor radiopacificador para Biodentina, que aumentou a radiopacidade e potencial bioativo enquanto manteve o tempo definido e a resistência à compressão em níveis aceitáveis como indica a norma ISO.

PALAVRAS-CHAVE

Trifluoreto de itérbio; Radiopacidade; Biodentina; Bioatividade.

INTRODUCTION

Biodentine ™ (BD, Septodont, Saint-Maur-des-Fosses Cedex, France) is a commercially available calcium silicate-based product. It was specifically designed as a "dentine replacement" material. In addition, it has various applications including; endodontic repair (root perforations, apexification, resorptions, and retrograde filling material in endodontic surgery) and pulp-capping agent.

Basically, Biodentine is formulated using the MTA-based cement technology [1] but with much shorter setting time, better mechanical and handling properties [2].

During endodontic treatment; evaluation of some clinical procedures as root canal treatment, detection of marginal defects, interfacial gaps or voids in root fillings, requires radiographic examination to verify the technical quality of treatment. Therefore, radiopacity, is considered an important property expected from a retrograde or a repair material as they need to be easily differentiated from the surrounding anatomic structures. Materials with low radiopacity may lead to misinterpretation of marginal gaps which may require retreatment with additional cost, chair time and patient discomfort [3].

One of the major drawbacks of the Biodentine was the reduced radiopacity and hence, inadequate radiographic visibility [4-7]. Several studies have shown that the radiopacity of Biodentine to be lower than other repair materials tested (MM-MTA, and MTA Angelus) and slightly lower than the 3 mm Al baseline value set by the ISO standards 6876:2012 [6].

Elements of high atomic number have been used in dentistry as radiopacifying agents, such as Ytterbium Tri-Fluoride. Ytterbium is an element of the lanthanide series, with a high atomic number (z = 71) and a refractive index of approximately 1.5 when forming a fluoride glass. Also, ytterbium trifluoride (YbF_3) was shown to be a suitable radiopacifying agent for Portland cement with no significant adverse effect on its mechanical properties [7].

Accordingly, the objective of this study was to evaluate the effect of adding Ytterbium Trifluoride on the radiopacity of Biodentine. The null hypothesis tested was that there is no difference in the radiopacity, compressive strength, setting time and bioactivity of Biodentine and the Ytterbium Tri-Fluoride modified Biodentine.

MATERIAL AND METHODS

The materials used in this study included: Biodentine™ (BD, Septodont, Saint-Maur-des-Fosses Cedex, France, lot no. B23276), Ytterbium Tri-Fluoride (anhydrous, powder, 99.98% trace metals basis, Sigma Aldrich, Buch, Germany, lot no. MKBW7519V) and Hank's Balanced Salt Solution HBSS (Lonza Walkersville, Verviers, Belgium).

Specimens' preparation and grouping

Biodentine specimens were prepared for each experimental condition and divided into four groups according to the concentration of YbF_3 added. Group X0 included the unmodified Biodentine that was mixed according to the manufacturer's instructions and acts as a control. Group X1 was prepared by the addition of 2.5% (0.017gm) YbF_3 into the Biodentine capsule. Group X2 was prepared by the addition of 5% (0.03 gm) YbF_3. Group X3 was prepared by the addition of 7.5% (0.052 gm) YbF_3. The three modified groups (X1, X2 and X3) were weighed by a digital scale (Sartorius, Cubis®, Germany) and added to the Biodentine capsule. Mechanical mixing for 30 seconds was done using amalgamator (3MCapmix™, 3M ESPE, Germany) to ensure homogenous distribution of the YbF_3 particles within the powder. Five drops of the liquid were then added to the powder capsule and the cement was mixed in the amalgamator

at 4650 rpm for 30 seconds according to the manufacturer's instructions.

Radiopacity testing

The radiographic test was performed in accordance with the ISO standards (6867:2012) for root canal sealing materials [6]. Biodentine was prepared according to the manufacturer's instructions, filled into ten cylindrical stainless-steel rings (10 mm in diameter, 1 mm thick), and preserved at 37º C for 24 hours. The specimens were radiographed by placing them directly on a photo-stimulating phosphor (PSP) plate (Sopro imaging, Acteon, USA) adjacent to an aluminum step wedge (purity at least 98% aluminum with a maximum copper content of 0.1% and maximum iron content of 1%) with thickness ranging from 0.5 to 9 mm; 0.5 mm for every increasing step. A standard X-ray machine (XGENUS De Götzen X-ray machine, Italy) was used to irradiate X-rays onto the specimens using an exposure time of 0.80s at 10 mA, tube voltage at 65 ± 5 kV and a cathode-target film distance of 300 ± 10 mm. A digital image of the radiograph was obtained. The gray level of each step in the step-wedge and of the cement was determined. The gray levels of the digital images were analyzed by imageJ software (LOCI, university of Wisconsin, Madison, USA) and the thickness of Al equivalent to the radiopacity of the cement had been estimated by incorporation of data into an equation following Couture and Hildebolt [8]. The grey pixel values of the cement specimens were determined and the relevant thickness of aluminum was calculated.

Compressive strength testing

Compressive strength was evaluated for the different groups according to the ISO standards (9917-2:2017) for water-based cements [9]. Ten cylindrical specimens (4 mm diameter and 6 mm height) for each experimental group were prepared using split Teflon mold. The cement was loaded into the split mold placed on a glass slide and covered with a celluloid strip and another glass slide from the top surface. After specimens' retrieval from the mold, it was inspected under 4 X magnification (HEINE® HRP 4X High Resolution Prismatic Binocular Loupes, Germany) for any voids or irregularities and stored at 37ºC in 100% humidity for 24 hours before testing. The specimens were loaded vertically using the universal testing machine (LLOYD™ LR5K, USA) at cross head speed of 1mm/min till fracture. The compressive strength was calculated automatically using Bluhill3® software applying the following Equation 1:

$$C = 4P / \pi d^2 \quad (1)$$

Where P is the maximum load and d is the specimen diameter.

Assessment of setting time

Setting time was assessed for the different experimental groups (n=10 for each group) according to the ISO standards (6867:2012) for root canal sealing materials [6]. The setting time measurement was carried out using rounded indenters of (95±5 gm and 2mm tip diameter) for the initial setting time and (400± 5 gm and 1 mm tip diameter) for the final setting time. After mixing, the cement was applied into a split circular Teflon mold (10 mm diameter x 2mm thickness). The indenter was lowered vertically to the surface of the cement and allowed to remain stable for 5 seconds. The indentation was repeated every 30 seconds till the needle fails to make a complete circular indentation in the cement surface. The time elapsed from the end of mixing till the tip fails to indent the cement surface was recorded to determine the setting time.

IN VITRO BIOACTIVITY

Specimens' preparation

Biodentine ™ capsules from the different experimental groups; (X0, X1, X2, X3) were mixed according to the manufacturer's instructions. The fresh material paste was packed into a split Teflon molds (10 ± 0.1 mm diameter and 2 ± 0.1 mm thickness) placed on a clean glass slide and the top surfaces was covered with a celluloid strip and glass slide and left undisturbed till hardening. Material discs (n = 10 for each experimental group) were immediately immersed in 10 mL of HBSS (Hank's Balanced Salt Solution) in a sealed 50 mL sterile CELLSTAR® polypropylene tubes (Greiner Bio One International, GmbH, Germany) and stored in the incubator at 37 °C.

Evaluation of bioactivity (apatite forming ability)

ESEM / EDX assessment:

The discs were collected at three endpoints (1, 7, and 28 days).The surfaces of the specimens were examined using an environmental scanning electron microscope (ESEM) INSPECT-FEG (Field Emission Gun) connected with EDX Unit (Energy Dispersive X-ray analysis), with accelerating voltage 30 K.V., and using 14x up to 1,000,000x magnification. The discs were surveyed and examined at different magnifications and elemental analysis of the different observed phases was performed using EDX unit. After each examination interval, the discs were returned into a fresh 10 mL of HBSS and stored in the incubator up to the next examination interval.

XRD analysis

After the final ESEM and EDX analysis (28 days), the discs were crushed using mortar and pestle to form a fine powder for XRD analysis to detect the different crystalline phases formed after hydration of the Biodentine™ and storage in the HBSS for 28 days.

PH evaluation

The HBSS was collected at the 3 predetermined intervals (1, 7, 28 days) and the pH of the solutions was evaluated using pH meter (B712 LAQUA twin compact pH meter, Horiba Scientific, Japan). 1 mL from each storage polypropylene tubes was aspirated using micropipette and placed in the pH meter measuring lens after being calibrated with the neutral calibration solution (pH 7). The resultant pH readings were recorded and compared to a fresh HBSS pH.

Statistical analysis

Numerical data were explored for normality and variance homogeneity using Shapiro-Wilk and Leven's tests respectively. Data showed parametric distribution and homogeneity of variances across groups so, they were represented as mean and standard deviation (SD) values and were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.0.3 for Windows [10].

RESULTS

Radiopacity

Radiographic images obtained with digital radiography system (Sopro imaging software, Acteon, USA) showing the specimens of the modified Biodentine discs (X1, X2, X3) alongside with the unmodified Biodentine (control) (X0) and the aluminum step wedge are showed in Figure 1.

Mean and standard deviation (SD) values of radiopacity for different groups are presented in Table I.

Results of one-way ANOVA test revealed a significant difference between different groups

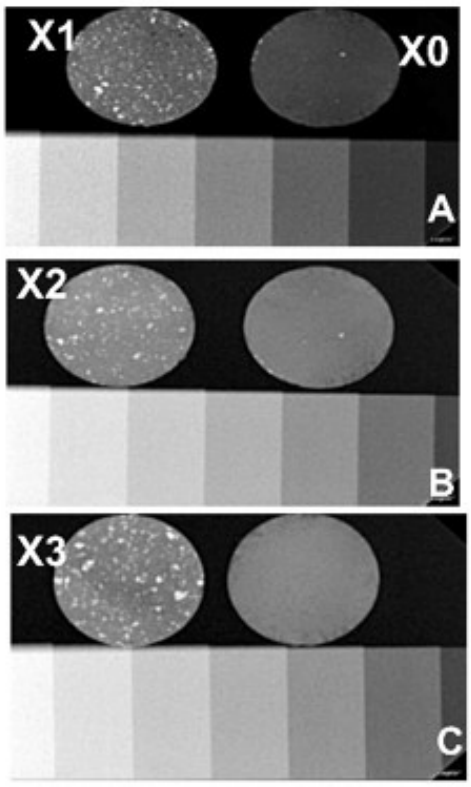

Figure 1 - Digital Radiography images showing the difference in radiopacity in (A) X1 group, (B) X2 group, (C) X3 group. Alongside with the unmodified Biodentine (X0) and the Aluminum step wedge.

Table I - Mean ± standard deviation (SD) of radiopacity for different groups

Radiopacity (mean ± SD)				p-value
X0	X1	X2	X3	
2.32 ± 0.17^{D}	3.00 ± 0.28^{C}	3.33 ± 0.12^{B}	3.64 ± 0.29^{A}	<0.001*

Means with different superscript letters within the same row are statistically significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

($p<0.001$). Post hoc pairwise comparisons showed values of different groups to be significantly different from each other ($p<0.001$).

Compressive strength (MPa)

Mean and standard deviation (SD) values of compressive strength (MPa) for different groups were presented in Table II.

Results of one-way ANOVA test showed that there was a significant difference between different groups ($p<0.001$). Post hoc pairwise comparisons showed X0 group to have a significantly higher value than other groups ($p<0.001$).

Setting time

Mean and standard deviation (SD) values of setting time (hr:min:sec) for different groups were presented in Table III.

Results of one-way ANOVA test showed that there was no significant difference between different groups ($p=0.074$) for the initial setting time.

Table II - Mean ± standard deviation (SD) of compressive strength (MPa) for different groups

Compressive strength (mean ± SD)				p-value
X0	X1	X2	X3	
67.85 ± 7.05^{A}	53.45 ± 2.84^{B}	52.56 ± 7.09^{B}	45.59 ± 1.13^{B}	<0.001*

Means with different superscript letters within the same row are statistically significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

For the final setting time, results of one-way ANOVA test revealed a significant difference between different groups ($p<0.001$). Post hoc pairwise comparisons showed X3 group to have a significantly higher value than X2 group where both groups showed significantly higher final setting time values compared to other groups ($p<0.001$). There was no significant difference between X1 and X0 groups ($p>0.05$).

pH results

Mean and standard deviation (SD) values of pH values for different groups were presented in Table IV.

Results of one-way ANOVA revealed no significant difference between different groups at day 1, day 7 and day 28 ($p=0.719$), ($p=0.075$) and ($p=0.132$) respectively.

There was a significant interaction between the time of measurement and concentration of radiopacifier ($p=0.004$). For all groups, repeated measures ANOVA showed there was a significant difference between values measured at different intervals ($p<0.001$) and post hoc pairwise comparisons showed values of different intervals to be significantly different from each other. Values increase with increasing time from Day 1 to Day 28 ($p<0.001$).

In vitro bioactivity (Apatite forming ability)

The results of ESEM analysis of the X0 group at day 1 showed a uniform surface containing interspersed granules and after soaking in HBSS

Table III - Mean ± standard deviation (SD) of setting time (hr:min:sec) for different groups

Setting time	Setting time (mean ± SD)				p-value
	X0	X1	X2	X3	
Initial	12:42±03:17	16:22±03:36	19:24±01:01	13:21±03:13	0.074
Final	$0:31:46\pm0:05:45^{C}$	$0:44:45\pm0:07:03^{C}$	$1:07:03\pm0:13:04^{B}$	$1:28:56\pm0:15:03^{A}$	<0.001*

Means with different superscript letters within the same row are statistically significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).

Table IV - Mean ± standard deviation (SD) of pH values for different groups

	pH value (mean ± SD)				P value
	X0	X1	X2	X3	
Day 1	7.82±0.02Ac	7.81±0.08Ac	7.90±0.20Ac	7.88±0.20Ac	0.719
Day 7	8.61±0.03Ab	8.65±0.07Ab	8.70±0.11Ab	8.61±0.06Ab	0.075
Day 28	11.80±0.19Aa	11.90±0.14Aa	11.90±0.20Aa	11.56±0.38Aa	0.132
p-value	<0.001*	<0.001*	<0.001*	<0.001*	

Means with different upper and lowercase superscript letters within the same row and column respectively are statistically significantly different *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

for 7 days a layer of globular CaP precipitates formed on the surface Figure 2. This amount of surface layer has progressively increased after 28 days soaking in HBSS and this was confirmed by EDX analysis Figure 3. For X1, X2 and X3 groups, ESEM analysis showed progressive formation of superficial spherules on the surface of the Biodentine™ disc with the densest apatite layer formed on the surface of X1 group at different time intervals Figure 4. This was supported by EDX analysis which showed the highest peak of Ca (58.54 wt %) at 28 days Figure 5C. Numerous white crystals were dispersed throughout the set cement denoting the ytterbium radiopacifying agent in X1, X2 and X3 groups Figures 4, 6 and 8. This was confirmed by EDX spectrum which showed peaks of Ytterbium and small peaks of Fluoride Figures 5, 7 and 9 at 28 days, the deposition of CaP layer increased and became rich on the surface in the form of petal-shaped crystals in X1 and X2 groups Figures 5 and 7. XRD analysis of all groups showed that during hydration, calcium silicate phases transformed into calcium carbonate (calcite), calcium silicate hydrate (tobermorite) and amorphous calcium hydroxide (portlandite). X1 group showed the highest Portlandite phase of all groups and other phases were similar to X0 group Figure 10A, b while X3 group showed the lowest phases of all groups Figure 10D. Calcium ytterbium fluoride and ytterbium-III-oxide phases were detected in X1, X2 and X3 groups Figure 10B, C, D.

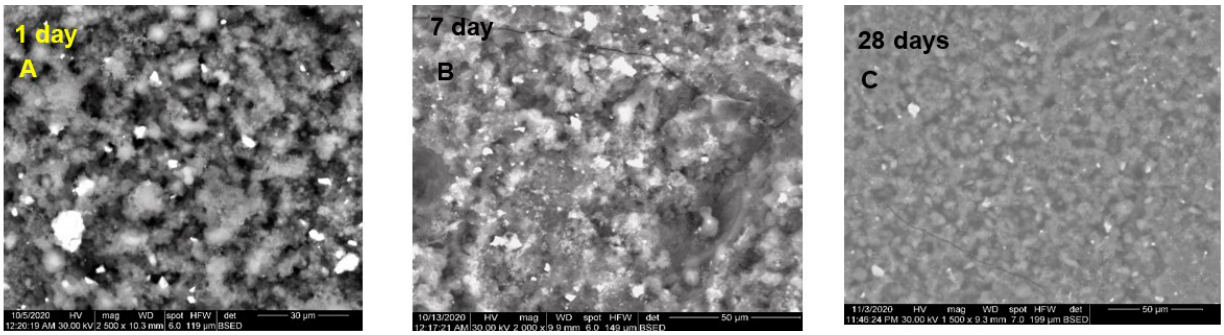

Figure 2 - ESEM photomicrographs for the X0 group stored in HBSS for different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

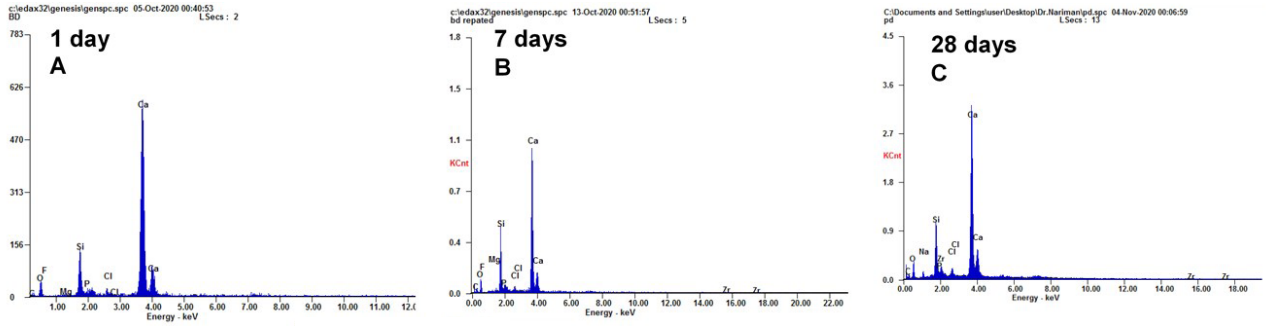

Figure 3 - EDX analysis for the X0 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

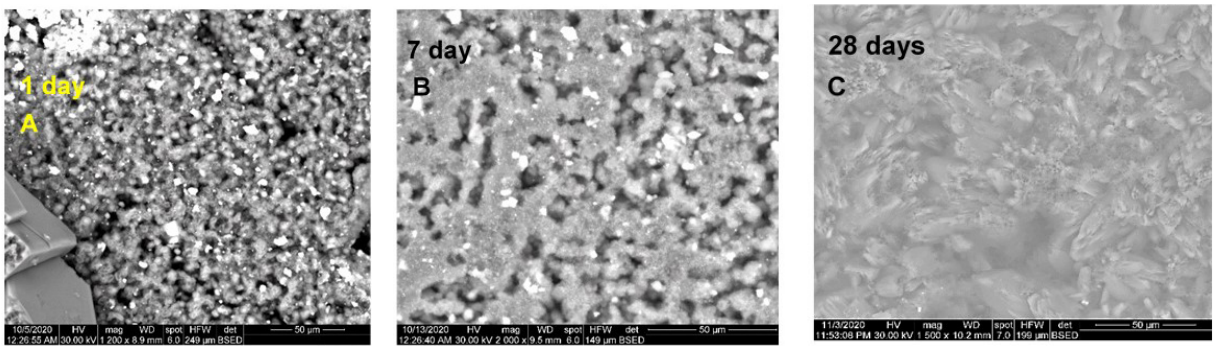

Figure 4 - ESEM photomicrographs for the X1 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

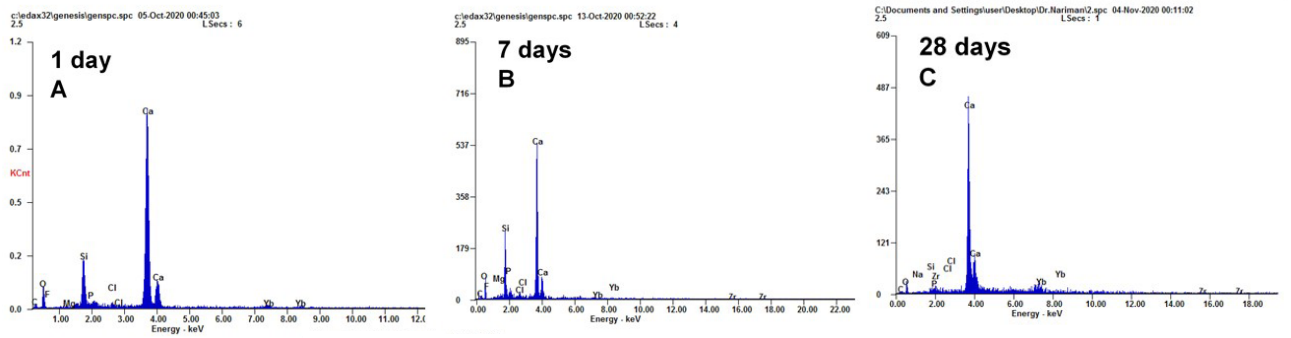

Figure 5 - EDX analysis for the X1 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

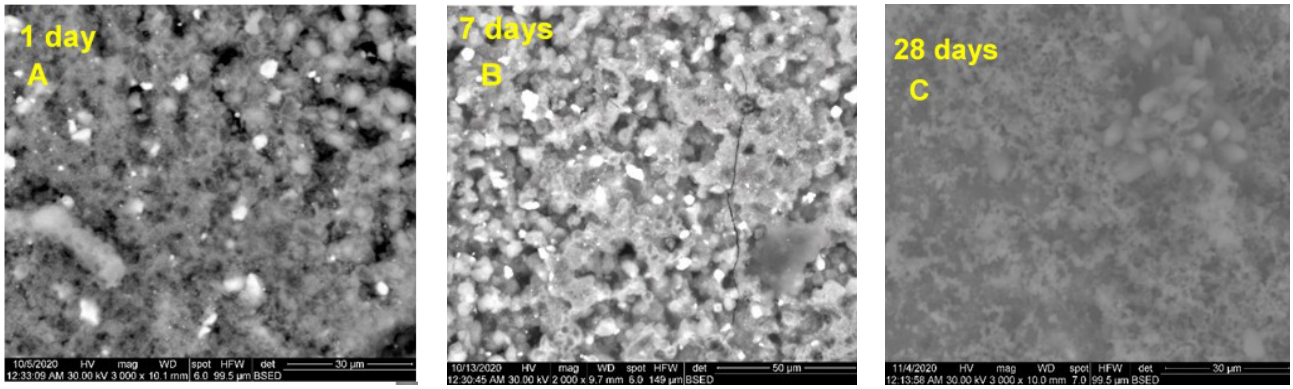

Figure 6 - ESEM photomicrographs for the X2 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

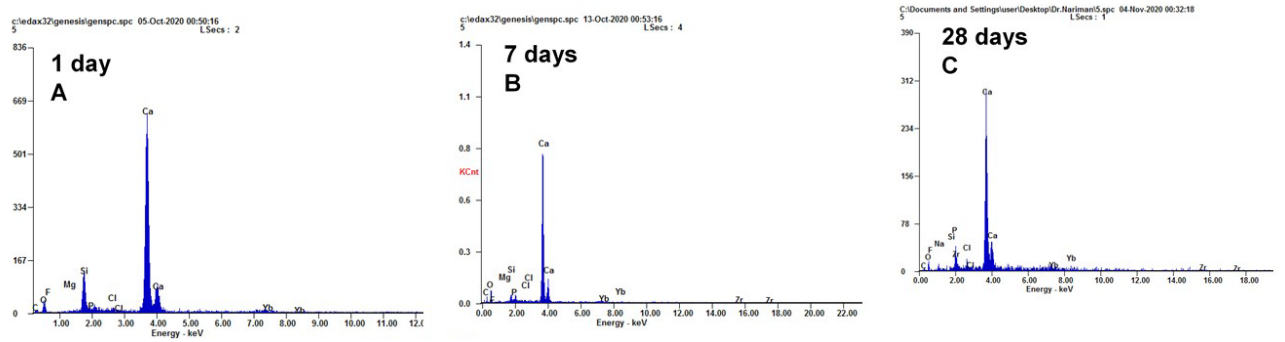

Figure 7 - EDX analysis for the X2 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

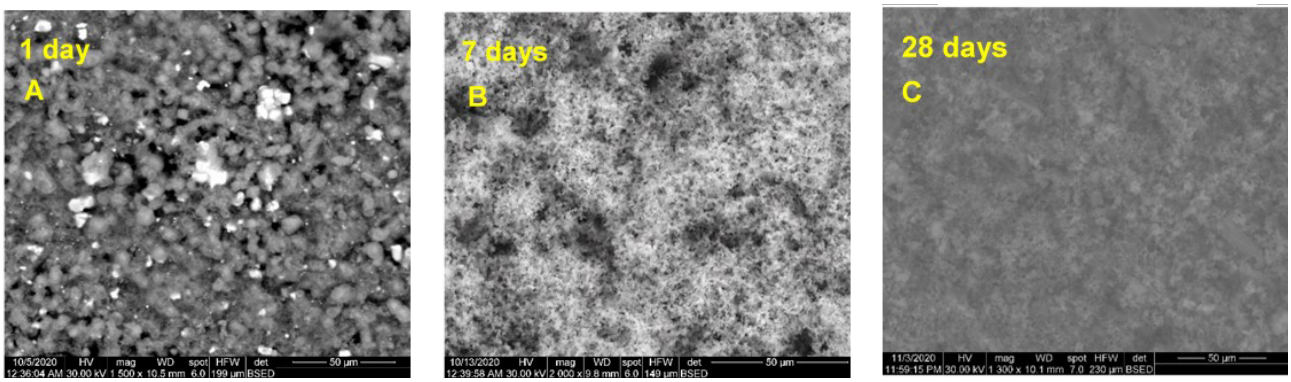

Figure 8 - ESEM photomicrographs for the X3 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

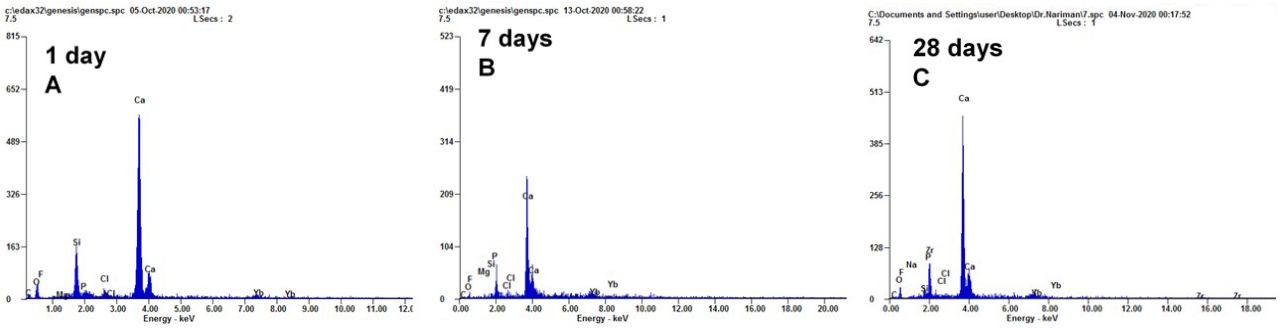

Figure 9 - EDX analysis for the X3 group stored in HBSS for the different time intervals: (A) Day 1, (B) Day 7 and (C) Day 28.

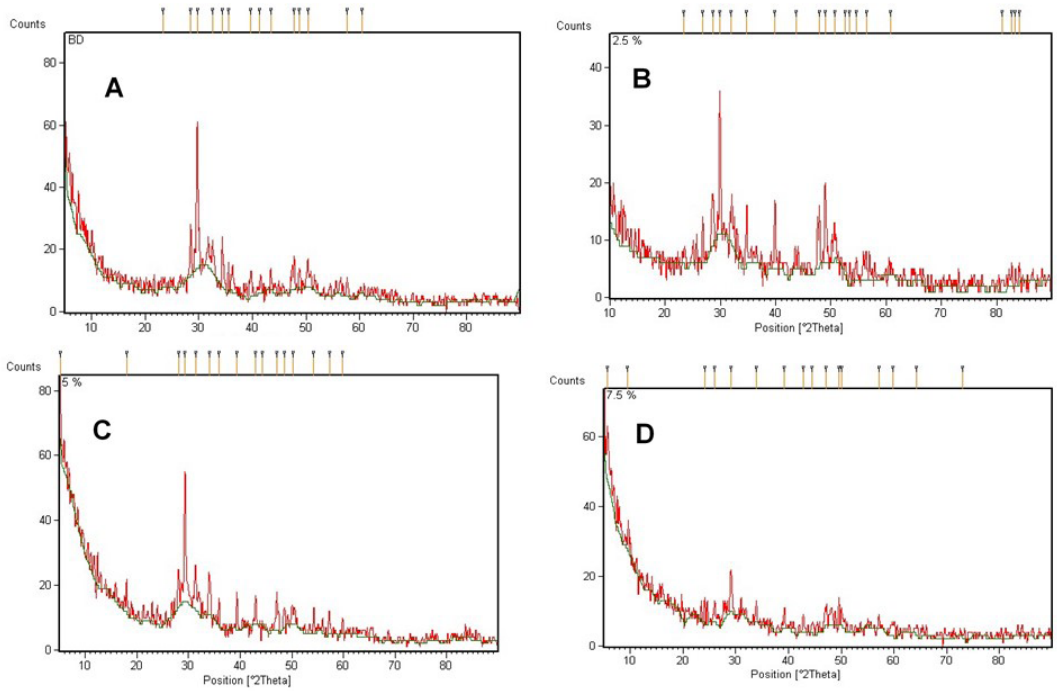

Figure 10 - XRD analysis for the crushed X0, X1, X2 and X3 cement groups in A, B, C and D respectively after storage for 28 days in HBSS.

DISCUSSION

One of the Major disadvantages of Biodentine was its relatively low radiopacity [4,5]. Unfortunately, limited studies were conducted in an attempt to improve the radiopacity of Biodentine. In 2019, Ochoa-Rodríguez [11] modified Biodentine with 15% Calcium Tungstate or 15% Zirconium Oxide and reported a significant improvement in its radiopacity up to values higher than 3 mm Al recommended by the ISO 6876 [6]. Different radio-opacifiers were used by researchers to modify the radiopacity of dental cements. Ytterbium Tri-Fluoride was used in many studies as a radio-opacifying agent [12,13]. Ytterbium has a high atomic number (Z=70) which is much higher than the Zirconium (Z=40) in Zirconium Oxide used in Biodentine [14]. Thus, it was of great interest to evaluate the effect of addition of YbF_3 to Biodentine™. In this study, YbF_3 powder was added to Biodentine in 2.5%, 5% and 7.5%. A pilot study was performed and it was found that minimum 2.5% of YbF_3 has proved sufficient radiopacity. The YbF_3 powder was added to the Biodentine powder in the capsule and was mechanically mixed in the amalgamator for 30 seconds to ensure homogenous distribution [15,16].

For radiopacity assessment, the ISO standards 6876-2012 for root canal sealers were applied [6]. A 0.5 mm-increment of the Aluminum step wedge was used instead of a standard 1.0 mm-increment to provide more details and accuracy. In this study, the mean

radiopacity for the Biodentine after the addition of 2.5%, 5% and 7.5% YbF_3 has significantly increased with increasing YbF_3 concentration ($p<0.001$). The degree of radiopacity of any particle depends on the atomic number of its components in addition to their density and size. Elements with high atomic numbers can absorb or reflect light, thus affecting light scattering within the material making it radiopaque [17,18]. Ytterbium particles in YbF_3 used in this study have a high atomic number (Yb=70) which is much higher than Zirconium (Zr=40) in ZrO_2 used in Biodentine and that's why smaller amount of Ytterbium would produce higher radiopacity than Zirconium.

For assessment of the setting time, ISO standards (6876) for root canal sealers [6] were applied as they evaluate both initial and final setting times for the same material disc. The addition of YbF_3 particles to Biodentine did not significantly affect the initial setting time ($p=0.074$). This indicates that YbF_3 particles have been incorporated into the hydration reaction of Tri Calcium Silicates to release Calcium Silicate hydrate and Calcium hydroxide. Regarding the final setting time, the addition of 2.5% YbF_3 had no significant difference to the unmodified group. However, with higher concentrations (5% and 7.5%), the final setting time has been significantly extended ($p<0.001$). It could be suggested that the low concentration of YbF_3 used (2.5%) could be incorporated into the Tri-Calcium Silicate hydration reaction however, higher concentrations (5% and 7.5%) may have led to inhibition of the hydration reaction and prevention of cement particle packing which in turn has prolonged the cement matrix formation [19]. The fact that the addition of radio-opacifiers extends or retards the setting time of the cements had been supported in many studies [11,19,20].

Regarding compressive strength evaluation, the addition of YbF_3 radio-opacifier to Biodentine had decreased the 24-h compressive strength. These findings were in line with Collares et al. [12], Prentice et al. [21] and Saghiri et al. [22]. The compressive strength values of the X1 group (53.45 MPa) and the X2 group (52.56 MPa) were found to be above the acceptance level for compressive strength of dental materials as determined by the 9917-2:2017 standards for water-based cement which is 50 MPa [9].

The in-vitro bioactivity assessment can rely on imaging the surface by SEM in conjunction with the semi-quantitative elemental analysis of the surface using EDX. This method gives the advantage of visualization of the surface deposits. ESEM analysis was performed instead of the conventional SEM to avoid any surface preparation or coating over the specimen before imaging which might cause changes in the surface chemistry. XRD enables quantitative characterization of the crystalline phases with different crystalline arrangement. pH analysis gives an indication about the alkalizing effect of the material due to Ca $(OH)_2$ elution which indicates apatite formation [23]. ESEM showed an evident globular surface layer deposit in all groups. This layer has been increasing significantly with increasing immersion time with petal-shaped crystals appeared in X1 and X2 groups after 28 days (Figures 4 & 6C). These petal shaped crystals suggested apatite formation [24]. The surface layer was denser and richer in X1 group than other groups for the same time interval. This was further supported by EDX analysis of X1 group which showed the highest Calcium release (52.96 At%) at 28 days Figure 5C. This was also reflected in the XRD analysis of X1 group which showed the highest Portlandite Ca $(OH)_2$ phase Figure 10B, while X3 group showed the lowest phases Figure 10D. This would further justify the suggestion that the 2.5%YbF_3 has been incorporated into the hydration reaction of Tri-Calcium Silicates into Calcium hydroxide and Calcium Silicate hydrate which filled up the pores leading to a dense structure. However, increasing the YbF_3 concentration up to 7.5% has led to inhibition of the hydration reaction and accordingly more porous structure appeared in ESEM photomicrographs Figure 8 as well as lower phases presented in the XRD Figure 10D.

The pH changes of HBSS after immersion of Biodentine™ discs showed potent alkalizing effect of the different experimental groups due to the continuous elution of Ca $(OH)_2$ as a result of the hydration reaction which was supported by the XRD results of the different experimental groups. The successive increase in pH with time denotes continuous formation and release of Ca $(OH)_2$ over extended period of time confirming the extended hydration and reactivity of the cement discs in HBSS. This assumption is also supported by the results of XRD that revealed presence of Ca $(OH)_2$ peaks even after immersion for 28 days in HBSS.

CONCLUSIONS

Based on the results obtained in this study, the null hypothesis tested was rejected as the addition of YbF_3 to Biodentine revealed significant differences in radiopacity, compressive strength and affected bioactivity as well. Hence, it could be concluded that 2.5%YbF_3 can be a promising radiopacifying agent to Biodentine with improvement in radiopacity and bioactive potential. In addition, the setting time was maintained and although there was a decrease in the compressive strength yet, still above the acceptable levels indicated by the ISO standards.

Authors' Contributions

NMB: corresponding author (conceptualization, methodology, software, formal analysis, investigation, writing - original draft). DIE: data curation, writing - review & editing, visualization, supervision, project administration. DIS: resources, writing - review & editing, visualization, supervision.

Conflict of interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted *in vitro* and did not involve any human or animal subjects. Therefore, the authors declare that the study does not require ethics committee approval

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Nareman Mahmoud Bahaa
(Corresponding address)
Egyptian Russian University, Faculty of Dentistry, Dental Biomaterials department, Cairo, Egypt
Email: Nareman.mahmoud11@hotmail.com

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