

ADDITION OF BIOACTIVE GLASS TO ENDODONTIC EPOXY RESIN SEALER: EFFECT ON BIOACTIVITY, FLOW AND PUSH-OUT BOND STRENGTH

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ABSTRACT

Objectives: The present study aimed to impart bioactivity to an endodontic epoxy resin sealer by incorporating bioactive glass nanoparticles (nBG) at two different concentrations and to study the effect of such modification on the sealer's flow and push-out bond strength with radicular dentin.

Materials and Methods: One type of endodontic epoxy resin sealer, AH 26, Dentsply, and 45S5 bioactive glass nanoparticles (nBG), purchased from NanoTech, Egypt, were used. Three sealer groups were investigated: a control group representing the AH 26 sealer without modification; groups (10%nBG) and (20%nBG) in which 10wt% and 20wt% nano bioactive glass was added to the sealer powder respectively. The bioactivity of the three sealer groups was assessed by immersing sealer discs into simulated body fluid (SBF) at 37 °C for two and four weeks (n=8). After each time point, the discs were retrieved from the immersion medium, dried and examined for the formation of calcified deposits using scanning electron microscope (SEM). Whenever surface precipitates were found, elemental surface analysis of the deposits was performed using Energy Dispersive X-ray spectroscopy (EDX). For each group, SEM examination was also performed on representative sealer discs without immersion into the SBF to be used as a reference. To measure the flow, a specified volume of the freshly mixed sealer was squeezed between two glass slabs under specified weight and the diameter of the formed sealer disc was measured as an indicator of the sealer's flow as recommended by ISO (n=5). For measuring the push-out bond strength, nine extracted sound human upper central incisors were used. The teeth were endodontically treated and obturated by gutta percha in combination with one of the three investigated sealer compositions. After incubation in SBF for two weeks, the roots were horizontally sectioned into 2-mm-thick slices. The push-out bond strength was then measured using a universal testing machine (n=9).

Results: After four weeks, the 10%nBG group showed *in vitro* bioactivity and formed calcified Ca-P rich deposits that had Ca/P ratio in the range of that of biological hydroxyapatite. On the other hand, the 20%nBG groups formed calcium phosphate deposits whose Ca/P ratio did not lie within the hydroxyapatite range. The 20%nBG group exhibited lower flow than the 10%nBG group and the control but remained acceptable according to the ISO standards. The push-out bond strength did not differ significantly among the three groups.

Conclusions: The incorporation of 10wt% nano bioactive glass imparts bioactivity to the resin sealer without adversely affecting the sealer's flow or push-out bond strength.

KEYWORDS: Epoxy resin sealer; Bioactive glass nanoparticles; Bioactivity; Flow; Push-out bond strength

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INTRODUCTION

The success of endodontic treatment requires hermetic three-dimensional obturation of a properly cleaned and shaped radicular space ^(1,2). For decades; although not ideal, gutta percha combined with a sealer has been considered the gold standard of root canal filling materials ⁽¹⁻³⁾. Root canal sealers serve as space fillers for any discrepancies between the root canal wall and the core filling material. Consequently, they play an important role in achieving an impervious seal. Among the requirements of an ideal root canal sealer is its ability to form a fluid-tight seal; apically, laterally and coronally, between the dentin and gutta percha. To achieve this, the sealer must have adequate bond strength to both the radicular dentin and the core material ⁽⁴⁾. Therefore, attention has been focused on the adhesive properties of root canal sealers.

In the early 2000s, with the introduction of adhesive dentistry in the field of endodontics, a new generation of polymer-based endodontic sealers capable of bonding to radicular dentin has become widely available ^(3,5). Resin-based sealers form a hybrid layer and, owing to their hydrophilic properties, penetrate deep into the dentinal tubules ⁽³⁾. Their retention relies on the contact between the sealer and the root dentin ⁽⁶⁾. Resin sealers are available as either epoxy or methacrylate-based products ⁽³⁾. Epoxy resin-based sealers gained wide acceptance in endodontic practice due to their favorable properties such as their long working time, ease of mixing and good sealing ability ^(4,7). They demonstrate excellent bonding to dentin due to their ability to chemically interact with collagen ^(8,9). However, the epoxy resin sealers lack the bioactivity associated with another sealer group, namely the bioceramic sealers ^(7,10)

The bioactive material is defined as “the material that elicits a specific biological response at its interface which results in the formation of a bond between the tissue and the material”. The bioactivity

of bioceramic root canal sealers allows them to precipitate apatite crystalline structures, which increase over time, upon exposure to phosphate-buffered saline. These precipitates account for the chemical bond between the sealer and the root dentin ⁽¹¹⁾. However, bioceramic sealers are difficult to remove in cases of retreatment and they also have flow characteristics higher than that recommended by ISO 6786/2001 ^(10,12). Thus, there has been an urge for alternative or modified sealers that both bond to the root dentin and filling materials, to achieve the monoblock concept, and meanwhile exhibit suitable flow characteristics ⁽¹⁰⁾.

Following its introduction by Prof. Hench, Bioglass 45S5 (BG) has been used by several researchers ⁽¹³⁾. BG is a type of glass that bonds to hard or soft tissue and possesses an antimicrobial activity ⁽¹⁴⁾. Therefore, bioactive glass-based materials have been introduced as promising hard tissue regenerative materials in the dental field. This was attributed to their apatite-forming ability as well as their ability to promote biomineralization of hard tissues i.e. bone, enamel, dentin, and cementum ⁽¹⁵⁾. Khvostenko *et al.* ⁽¹⁶⁾ investigated the effect of adding BG at different concentrations to resin composite on its mechanical properties. The authors reported that all BG-containing composites exhibited superior mechanical properties compared to the unmodified composite ⁽¹⁶⁾. Heid *et al.* ⁽⁷⁾ incorporated flame-sprayed bismuth oxide doped nanometric 45S5 bioactive glass and pure 45S5 micrometric bioactive glass into a commercially available epoxy resin sealer and evaluated their effect on the sealer radiopacity, microhardness, pH and mineral induction. It was reported that the nanometric 45S5 bioactive glass-containing sealer was more radiopaque, had quicker pH induction and Ca/P precipitation. Both investigated sealers had increased microhardness compared to the control ⁽⁷⁾.

Based on the previous review, it would thus be of interest to combine the proven advantages of an epoxy resin sealer with some desired properties of bioactive glass; particularly bioactivity. Therefore,

the aim of the current study was to evaluate whether incorporating nano-sized particulate 45S5 bioactive glass (nBG) into a commercially available epoxy resin sealer would impart bioactivity to the sealer without compromising its flow and push-out bond strength. The null hypothesis was that there was no significant difference between the bioactivity of the conventional epoxy resin sealer and that of the sealer modified with the bioactive glass.

MATERIALS AND METHODS:

Materials:

One endodontic epoxy resin sealer was used, AH 26, Dentsply, Germany. Nano 45S5 bioactive glass (nBG) was purchased from NanoTech, Egypt.

Methods:

In vitro Bioactivity:

The bioactivity was tested using the standard *in vitro* method of prolonged immersion in simulated body fluid (SBF) followed by surface microscopic examination⁽¹⁷⁾. Three groups were investigated: the control group representing the epoxy resin AH 26 sealer without any modification, the 10%nBG group and the 20%nBG group in which 10wt% and 20wt% nBG was added to the sealer powder before mixing (i.e. 400 mg and 800 mg nBG to each 4 g sealer powder respectively). Teflon molds were used to prepare eight disc-shaped samples from each sealer group (1 cm in diameter and 2 mm in thickness) (n=8). One end of a thread of unwaxed floss was embedded at the periphery of each sample to facilitate handling. The discs were immersed in SBF inside glass tubes. The SBF was prepared following the procedure described by Kokubo and Takadama using [NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, Na₂SO₄, 1M-HCl and TRIS Buffer]⁽¹⁷⁾. The sealer discs were suspended in the SBF hanging from the lids of the glass tubes so that their surfaces did not touch the walls of the containers and were only in contact with the SBF. The volume of the SBF placed

in each glass tube was 11 ml to yield a volume/area ratio of ≈ 5 ml SBF for each cm² of the disc surface as recommended in literature⁽¹⁸⁾. Six discs from each sealer group were immersed in the SBF (three discs for each of the two investigated immersion periods; two and four weeks). At the end of each immersion period, the samples were retrieved from the SBF, dried and examined for the precipitation of calcified deposits using scanning electron microscope (SEM). Whenever surface precipitates were found, their composition was explored by surface elemental analysis using Energy Dispersive X-ray spectroscopy (EDX). For each group, SEM examination of the surfaces of two sealer discs was carried out without immersion in SBF to be used as a reference for the sealer's original surface morphology.

Flow:

The flow of the sealer was measured using the ISO method (ISO 6876:2012(E))⁽¹⁹⁾. Two glass slabs, 20 g each, were used. After mixing, a volume of (0.05±0.005) ml of the sealer was dispensed on one of the glass slabs using a graduated syringe. After three minutes from the beginning of mixing, the second slab was placed on top of the dispensed sealer. An additional weight of 100 g was then placed on the glass slab so that the mixed sealer was compressed under a total weight of 120 g to form a thin disc. After 10 minutes, the minimum and maximum diameters of the compressed sealer disc were measured. If the difference between the two diameters was less than 1 mm, the average of the two diameters was taken as the final measurement. If the diameters differed from each other by more than 1 mm, the test was repeated. Five acceptable test runs were made for each group (n=5).

Push-out bond strength:

Nine extracted sound human central incisors were used in the present study. The teeth were obtained from the outpatient clinic, Surgery Department, Faculty of Oral and Dental Medicine,

Cairo University, and were only used after taking the approval of the Ethics Committee. First, the teeth were hand-scaled to remove any soft tissue residues or hard deposits then were disinfected and stored in artificial saliva.

The teeth were decoronated and the roots received conventional endodontic treatment. A K-file #10 was used to determine the working length of each root canal. The file was inserted into the canal until its end was just seen through the apical foramen. The file was then brought out of the canal and the inserted length was measured. One millimeter was subtracted from this measured length to yield the working length. The root canals were then prepared by hand instrumentation using the step-back technique and 0.02 taper K-files up to a master apical file #40. The rest of the canal was prepared using K-files larger than the master apical file up to file #50. During instrumentation, the canals were intermittently irrigated using freshly prepared 5.25% sodium hypochlorite solution (NaOCl) and at the end of preparation, the smear layer on the radicular dentin was removed by flooding the canal by 15% EDTA solution for five minutes then the canal was finally washed with NaOCl. The canals were dried using paper points to be ready for obturation. The prepared roots were randomly allocated into three groups that received different obturation regimens; the first group was obturated with gutta percha combined with the unmodified sealer (control), the second group was obturated with gutta percha combined with the 10%nBG sealer whereas the third group was obturated with gutta perch and the 20%nBG sealer.

After obturation, the teeth were stored in SBF for two weeks at 37 °C under 100% humidity. The roots were then vertically embedded in self-cured acrylic resin. The embedded roots were horizontally sectioned perpendicular to the long axis of the obturated canals using a diamond disc under water cooling. The sectioned slices were 2 mm in thickness. Three root slices were obtained from each tooth, yielding nine replicas per group (n=9).

The push-out bond strength test was performed on the slices using a universal testing machine (Instron universal testing machine model 3345, England) equipped with special small-sized tips at a crosshead speed of 0.5 mm/min using a 500 Newton load cell. During testing, the root slices were placed such that the apical side faced the pushing tip whereas the coronal side faced downwards. This ensured that any convergence in the canal would not interfere with the test. Three sizes of pushing tips were used (0.5, 0.7 and 1mm). For each root slice, the largest possible tip size was used provided that it only made contact with the filling materials (the gutta percha and sealer) without contacting the radicular dentin surface.

Each sample was loaded until the first sign of dislodgment of the obturating material. The maximum load before failure was recorded then the push-out bond strength was calculated according to the following equation:

$$\text{Push-out bond strength} = \frac{\text{Maximum load (N)}}{\text{Area of adhesion (mm}^2\text{)}}^{(3)}$$

To calculate the area of adhesion, the diameter of the root space was measured from both the apical and the coronal sides. The area of adhesion was calculated as follows:

$$\text{Area of adhesion} = \pi (R_c + R_a) \times h$$

where R_c is the root radius as measured from the coronal side, R_a is the root radius as measured from the apical side and h is the slice thickness.

Statistical analysis:

The data were presented as means and standard deviation values. Data were explored for normality using the Shapiro-Wilk test. The one-way ANOVA test was used to compare between the different groups while the Tukey's HSD post-hoc test was used for pairwise comparison when the ANOVA showed significant difference. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

In vitro bioactivity

The SEM examination of the control group without immersion in SBF showed a relatively irregular surface. After two and four weeks of immersion, cracks and surface craters were seen all over the surface of the sample (Fig. 1). For the

10% nBG group, abundant surface porosities and marked deposition of Ca-P rich precipitates were observed after two weeks of immersion. EDX analysis of these precipitates revealed that they had a Ca/P atomic molar ratio of 1.21. The precipitates did not completely cover the nBG particles as evidenced by the appearance of the Si peak in the EDX spectrum. After four weeks of immersion in

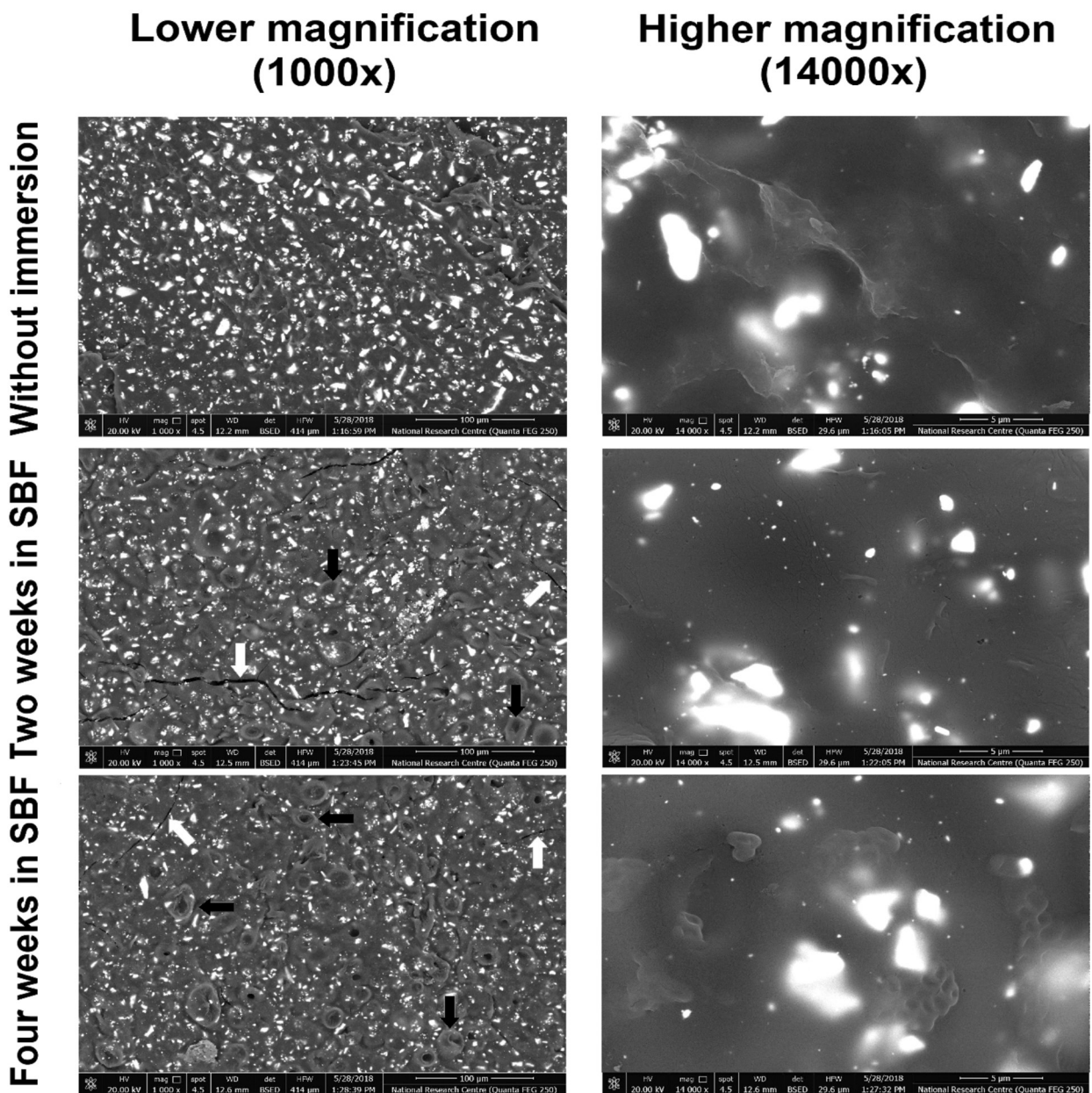


Fig. (1): SEM of the control group without immersion and after two and four weeks of immersion in SBF. The white arrows show the surface cracks while the black arrows mark the surface craters

SBF, the formation of the Ca-P rich precipitates was more intensive, the precipitates became more dense, well-developed and attained a globular highly porous morphology. The EDX results showed an increase in the Ca and P peak intensities and a decrease in the Si peak as compared to the two weeks results. In addition, the EDX showed that the Ca/P ratio of the surface deposits increased up to 1.61 after four weeks, which lies within the range of hydroxyapatite molar ratio ⁽²⁰⁾. (Fig. 2).

After both two and four weeks of immersion, the 20%*n*BG group showed more abundant calcified surface precipitates than the 10%*n*BG group. However, after two weeks of immersion, unlike the 10%*n*BG group which showed one form of deposits, the 20%*n*BG group showed the formation of two distinct morphologies of Ca-P rich deposits: calcified spongy patches and calcified nodules. Although the amount of the precipitates and their Ca/P molar ratio increased from week two to week four, the ratio still remained below the documented hydroxyapatite Ca/P ratio range ⁽²⁰⁾ (Fig. 3).

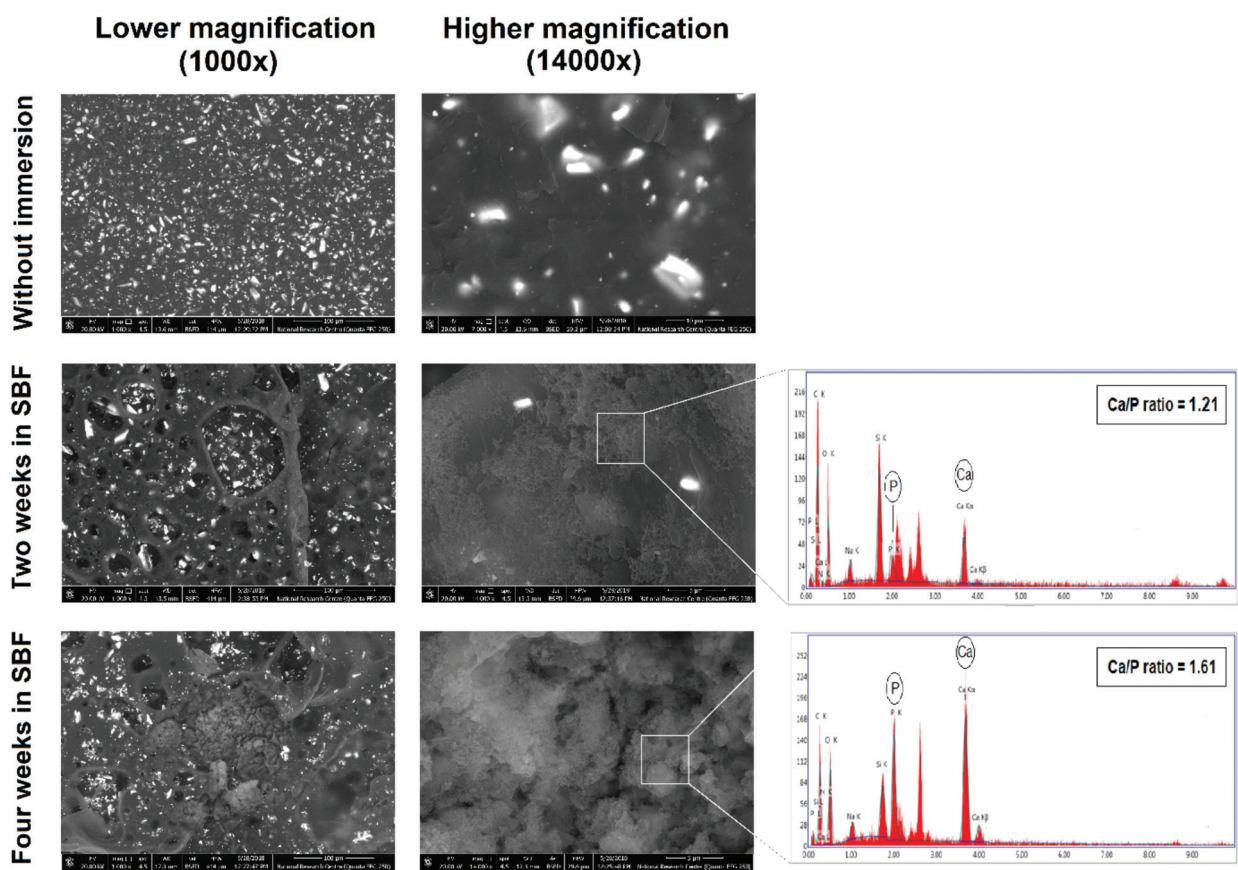


Fig. (2): SEM and EDX of the 10%*n*BG group without immersion and after two and four weeks of immersion in SBF

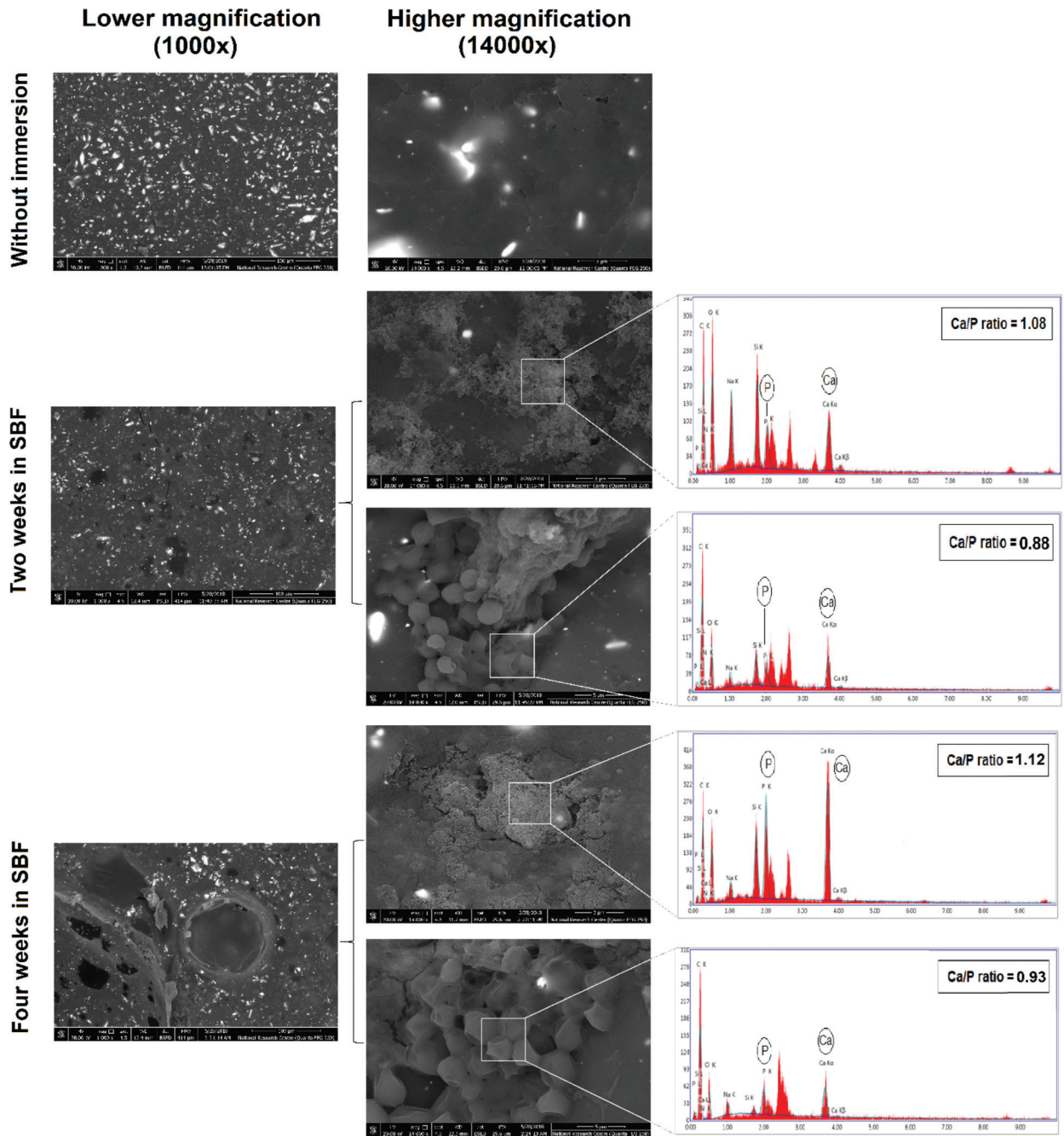


Fig. (3): SEM and EDX of the 20%nBG group without immersion and after two and four weeks of immersion in SBF

Flow:

No significant difference in flow was found between the control and the 10% nBG group. On the other hand, the 20% nBG had significantly lower flow compared to the other two groups (Fig. 4). However, its flow values were still higher than the minimum flow values accepted by the ISO standards for endodontic sealers⁽¹⁹⁾.

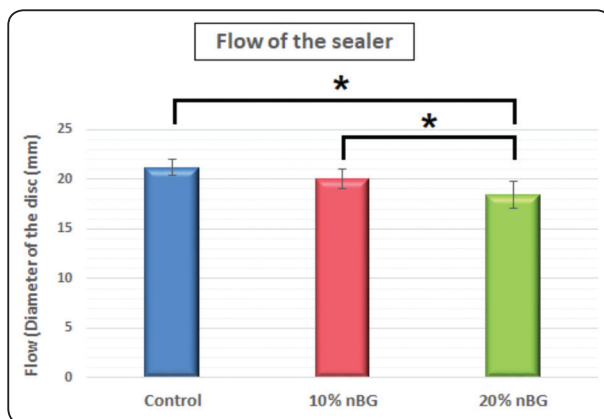


Fig. (4): The flow of the three sealer groups

Push-out bond strength

The statistical analysis revealed no significant difference in the push-out bond strength between the three investigated groups (Fig. 5).

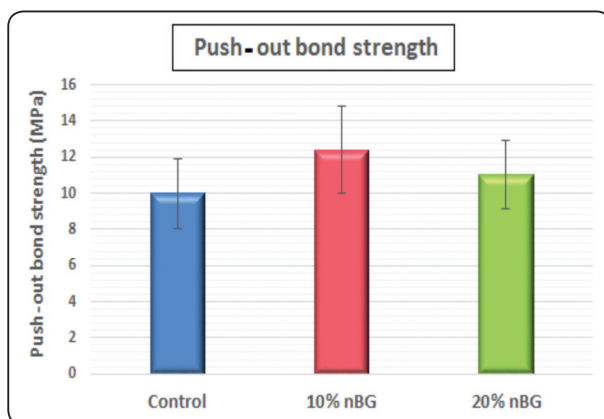


Fig. (5): The push-out bond strengths of the three groups

DISCUSSION

Examination of apatite formation on the material's surface after soaking in SBF is a valid well-accepted method for predicting the material's *in vivo* bioactivity⁽¹⁷⁾. SEM imaging coupled with surface elemental analysis using EDX spectroscopy is a valuable tool for examining the morphology of the precipitates formed on the bioactive surface and for identifying their composition. Generally, biological apatite found in calcified tissues is non-stoichiometric and the Ca/P ratio varies between enamel, dentin and bone⁽²¹⁾. A wide range of Ca/P ratios have been reported for each of the three tissue types. However, when it comes to judging bioactivity, Ca/P ratios ranging from 1.67 (stoichiometric) to ~1.5 (for calcium-deficient hydroxyapatite) is considered acceptable and indicative of bioactivity⁽²⁰⁾.

SEM examination of the control group after immersion in SBF for both two and four weeks showed the presence of many surface cracks and craters (Fig. 1). These surface features may be attributed to the hydrophilic nature of the sealer that may have resulted in liquid imbibition during immersion in the SBF and subsequent cracking while drying the samples before microscopic examination⁽³⁾.

After two weeks of immersion in SBF, the SEM of the 10% nBG group revealed the formation of Ca-P rich deposits that had a Ca/P ratio of 1.21 which is lower than the hydroxyapatite molar ratio. However, after four weeks, the deposits became denser and a decrease was observed in the peak intensity of Si, which is a main component of the bioactive glass implying that the densely precipitated formations obscured the underlying bioactive glass structure. The newly formed deposits had a porous globular morphology and covered a wider surface area of the sample and the Ca/P ratio raised to 1.61, which lies within the hydroxyapatite range, confirming a bioactive behavior (Fig. 2).

Based on these results, the null hypothesis was rejected. The increase in the Ca/P ratio between two and four weeks may be attributed to the fact that the deposition of hydroxyapatite on bioactive surfaces is a multi-phasic process that involves dissolution, precipitation and crystallization. It is already well-established that the hydroxyapatite formation is preceded by precipitation of amorphous calcium phosphate clusters whose Ca/P ratio lies in the range of 1.18-2.5. These clusters are not stable and undergo gradual crystallization and maturation into hydroxyapatite ⁽²⁰⁾. This trend of gradual increase of the Ca/P ratio of calcium phosphates precipitated on bioactive surfaces has been reported in previous research ⁽²²⁾.

Different results were observed for the 20%nBG group where more deposits were formed compared to the 10%nBG group. Two different shapes of calcium phosphate formations were observed; calcified spongy patches and nodular deposits. However, the Ca/P ratio of these formations did not match that of hydroxyapatite even after four weeks (Fig. 3). It may be inferred from these findings that increasing the concentration of the nBG in the sealer may affect the quantity of the formed precipitates (as evidenced by forming more deposits) but does not necessarily ensure attaining the required quality of the formed deposits (as evidenced by forming only non-apatitic precipitates). However, to confirm this inference, studies of longer immersion periods are needed. It is noteworthy that the deposits formed on the 20%nBG samples had much lower Ca/P ratios (from 0.88 to 1.12) than 1.67 and it has already been proven that the lower Ca/P ratio yields calcium phosphate compounds with higher solubility ⁽²³⁾. Thus, it is possible that the addition of 20% nBG; being of higher percentage, may have resulted in soluble deposits representing intermediate phases which, if given longer time, may have dissolved, re-precipitated and crystallized into mature hydroxyapatite.

Although imparting bioactivity to resin sealer may sound attractive, care should be taken that this optimization should not come at the expense of other sealer's critical properties such as the flow and the push-out bond strength. A sealer of adequate flow would be able to reach and seal narrow irregularities, lateral canals and the apical foramen. However, too high flow is also undesirable. In addition to the difficulty to control the material during placement into the canal, too high flow may also result in extruded material beyond the apical foramen, compromising periapical healing ⁽²⁴⁾.

The results revealed that the addition of 10wt% nBG to the epoxy resin sealer did not affect its flow while adding 20wt% decreased the flow, although within the range accepted by the ISO ⁽¹⁹⁾ (Fig. 4). This thickening effect may be explained by the nature of the nano-sized particles. Compared to micro-sized particles, nanoparticles, regardless of their composition, have a greater effect on increasing the viscosity of the polymer matrix in which they are embedded. This effect is attributed to their relatively larger surface area and greater surface energy which in turn allow more particle-matrix interactions ⁽²⁵⁾. Apparently, this effect on the viscosity would be dose-dependent because the higher the percentage of nanoparticles added to the polymer matrix, the more the surface interactions. Accordingly, that may explain why the addition of 10wt% nBG did not significantly affect the sealer's flow while the addition of 20wt% nBG did. Not only was the decrease in the flow of the 20%nBG group within the acceptable range but it also did not have an impact on the bond strength with radicular dentin where the 20%nBG group's push-out results did not significantly differ from the other two groups (Fig. 5).

One of the factors that contribute to the success of root canal treatment is the tenacious bond between the root canal sealer and the radicular dentin, and the bond between the root canal sealer and the obturating

material⁽²⁶⁾. Since a strong inverse correlation exists between root filling bond strength and leakage, thus the bond strength tests may be taken as a measure of effective filling of the root canal⁽²⁾. Compared to the conventional shear bond strength tests, the push-out test provides more reliable and reproducible results and allows a realistic assessment of the bond strength to dentin even at low values⁽⁸⁾.

Resin-based epoxy resin sealers are known to have superior bond strength compared to other types of sealers. This is attributed to the covalent bond formed between the open epoxide rings in the sealer and the amine groups in the dentin collagen network. This is in addition to the sealer's negligible (if any) polymerization shrinkage as well as its relatively long setting time which allows adequate penetration into the microspaces found between the dentin and the core material^(8,9,27).

In the present study, the addition of nBG epoxy resin sealers did not improve the push-out bond strength (Fig. 5). This may be attributed to the fact that the addition of nBG can act as a double-edged weapon. From one side, adding the nBG exerts a negative effect on the bond as it decreases the volume fraction of the resin in the sealer thus decreases the number of epoxide rings available for covalent bonding with the dentin collagen. On the other hand, the nBG bio-activates the sealer and promotes the deposition of calcium phosphate precipitates at the dentin-sealer interface. It has been previously proposed that the minerals formed by bioactive sealers may get deposited within the collagen fibrils forming interfacial tag-like structures that strengthen the interfacial bond⁽²⁸⁾. It is the balance between these negative and positive effects that may have resulted in the non-significant effect on the sealer's bond strength upon nBG addition.

It should be noted that for any added particles to exert a strengthening effect on a polymeric matrix, surface silanization should be made to

strengthen the particle-matrix bond and to allow for load-sharing at the interface between the two components⁽¹³⁾. However, in the present study, unsilanized nBG particles were used because it was assumed that the silane coating, if used, would have concealed some of the glass particles thus would have interfered with the surface reactions involved in the bioactivity.

CONCLUSION

Within the limits of the current study, it can be concluded that incorporating 10wt% bioactive glass nanoparticles into an epoxy resin endodontic sealer is an effective way to induce bioactivity without compromising the sealer's flow or its push-out bond strength with radicular dentin. However, the addition of 20wt% bioactive glass nanoparticles did not induce hydroxyapatite formation within the time limit of the present investigation.

Conflict of interest

The authors have no conflict of interest to disclose.

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