BMC Oral Health

https://doi.org/10.1186/s12903-025-07439-4

Article in Press

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Received: 4 September 2025

Accepted: 26 November 2025

Published online: 05 December 2025

Cite this article as: Fidan Spor R., Zengin Kurt B., Balkan E. *et al.* Evaluation of the remineralisation effect and biocompatibility of boron-doped bioactive glass material on artificial root surface caries. *BMC Oral Health* (2025). https://doi.org/10.1186/s12903-025-07439-4

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Evaluation of the Remineralisation Effect and Biocompatibility of Boron-Doped Bioactive Glass Material on Artificial Root Surface Caries

Running title: Remineralisation of Bioactive Glass

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ABSTRACT

Background. Root surface caries, which frequently occur due to ageing and periodontal procedures, often require minimally invasive approaches, especially in molar teeth where access is limited. This study aimed to analyse the remineralisation effects and biocompatibility of experimental bioactive glass-containing materials modified through various boron concentrations on root surface caries.

Methods. For the synthesis of the experimental bioactive glass, a rapid alkali-mediated sol-gel was applied. H□BO□ was included in the composition for boron modification. Forty caries-free human molar teeth were used. Artificial root caries were formed after 15 days of incubation with a demineralisation solution. The teeth were randomly divided into four groups (n=10) as GroupF: Fluoride varnish, GroupBG: Experimental bioactive glass, GroupBG-7: Experimental bioactive glass modified with 7% boron and GroupBG-14: Experimental bioactive glass modified with 14% boron. Microhardness and laser fluorescence measurements were conducted for each sample at baseline, after demineralisation and after treatment. The SEM-EDS analysis was performed. The cell viability was evaluated after 24 and 48 hours of incubation using the human dermal fibroblast cell line (CCD-1072Sk) and the WST-1 viability assay. For statistical analyses, ANOVA, Tukey, Bonferroni, and one-sample t-tests were used ($p < 0.05$).

Results. No statistically significant differences were detected among the groups in terms of microhardness. Laser fluorescence measurements showed a significant difference in Group BG-14 compared to other groups. The SEM-EDS indicated that Group BG-7 exhibited higher Ca/P ratios and favourable elemental distributions, with surface morphology comparable to fluoride-treated dentine. In the 24-hour cytotoxicity assay, fluoride was cytotoxic at all doses, while the experimental bioactive glass and boron-modified groups only showed cytotoxicity at high doses. Low doses of boron-modified bioactive glass promoted fibroblast proliferation. Boron-doped bioactive glasses significantly improved root surface remineralisation and supported fibroblast

viability, with 7% and 14% boron groups demonstrating enhanced mineral deposition and reduced cytotoxicity compared to fluoride.

Conclusion. The study revealed that all treatment groups significantly improved root surface remineralisation. Boron-doped bioactive glass groups showed increased mineral deposition and reduced cytotoxicity compared to fluoride. These findings suggest that boron-doped bioactive glasses may offer a biocompatible alternative for the minimally invasive treatment of root caries.

Keywords. bioactive glass, biocompatibility, boron, cell viability, cytotoxicity, microhardness, remineralisation, root surface caries.

INTRODUCTION

Increasing awareness in health and improved access to oral healthcare services have contributed to a decline in edentulism and the preservation of natural teeth, particularly among the elderly population in industrialised countries [1]. Nevertheless, age-related periodontal changes—often associated with factors such as vigorous tooth brushing or periodontal disease— can lead to an increased exposure of root surfaces, which are more susceptible to the development of caries. Consequently, a rise in the incidence of root caries among older adults has been reported worldwide [2-4]. Root caries tend to progress more rapidly compared to enamel caries due to the dentine's higher solubility [5], and limited access to restorative treatments often necessitates the removal of substantial amounts of sound tooth structure [6].

Currently, fluoride application is the most widely used remineralisation method for the prevention of dental caries. Sodium fluoride (NaF) varnish is among the most concentrated fluoride products on the market. Although NaF varnishes may differ in composition and delivery systems, the majority contain 5% NaF, corresponding to 22,600 ppm fluoride. Due to its adhesive characteristic, NaF varnish can remain on the tooth surface for several hours. It is

among the most commonly used topical fluorides for caries prevention. In addition to preventing caries, NaF varnish is also used by dental professionals to arrest the progression of existing lesions. In a recently conducted systematic review, it has been concluded that 5% NaF varnish (22,600 ppm fluoride) is effective in arresting enamel caries [7]. Nevertheless, its efficacy in arresting dentine caries remains uncertain. Unlike enamel lesions, which occur in a highly mineralised tissue, dentine caries is more complex due to the structural characteristics of dentine containing nearly 50% organic material and water volumetrically [8]. The efficacy of fluoride in remineralisation therapies has been well documented in numerous studies and is considered the gold standard. Nonetheless, due to potential side effects and growing concerns regarding cytotoxicity, there is an increasing need to develop alternative approaches for remineralisation treatments [9].

Dentine remineralisation using a functional collagen matrix at the initial and moderate stages of caries development appears to be a reasonable approach to avoid invasive restorative procedures and to preserve the remaining tooth structure, consequently. Accordingly, strategies regarding the biomimetic remineralisation for dentine have attracted significant attention for the treatment of both carious and non-carious lesions [10-12]. These approaches involve the inclusion of bioactive chemical agents capable of interacting with the collagen-rich dentine matrix, enhancing its biomechanical properties, preventing further demineralisation, and/or promoting remineralisation [13].

Bioactive glass particles typically possess a mesoporous structure, which makes them advantageous for use as drug delivery systems in dentistry. To enhance the bioactivity of bioactive glass and to increase its porosity, modification with boron can be employed. Considering its drug absorption capacity, the development of a mesoporous silica matrix with boron incorporation may offer improved efficiency in terms of absorption [14].

Due to their ability to bond with mineralised tissues and release therapeutic ions such as phosphate, calcium and boron, bioactive glasses have attracted increasing attention in dentistry [15]. In these contexts, biocompatibility with soft tissue cells, particularly fibroblasts, is essential, as these cells contribute to the integrity and repair of gingival and periodontal tissues. The interaction of fibroblasts with bioactive glass surfaces or their ionic dissolution products can influence cell viability, extracellular matrix production and proliferation, all of which are critical parameters for evaluating the suitability of these materials for clinical use [16,17]. Therefore, assessing the cytocompatibility of dental bioactive glasses through *in vitro* fibroblast viability assays is a key step toward optimising their composition for safe and effective therapeutic use in oral medicine.

Nevertheless, the low mechanical strength of bioactive glasses still poses a great challenge in applications. Accordingly, to modify the structural and textural properties of bioactive glasses, additional ions or oxides can be incorporated into the basic system of bioactive glass. B□O□ is considered a valuable modifier for mesoporous bioactive glasses as it improves hardness and abrasion resistance in silicate- and phosphate-based systems and has therefore been incorporated into melt-derived glass compositions. Beyond its mechanical benefits, bone is known to play key biological roles, such as supporting embryonic development, bone formation, and psychomotor performance. Adequate intake of boron compounds has been associated with the maintenance of bone health and enhanced compressive strength. In line with this, boron-doped bioactive glasses have shown improved biocompatibility and antibacterial effects, while also promoting osteoblast proliferation *in vitro*. Importantly, the controlled release of boron ions from such glasses can stimulate mineralisation processes and facilitate bone tissue regeneration [18-21].

This study aimed to analyse the effects of remineralisation and assess the biocompatibility of boron-doped bioactive glass materials and fluoride varnish (Duraphat®, Colgate-Palmolive, Germany) on artificially induced root surface caries.

The null hypothesis of this research is that there would be no significant difference in the remineralisation effects between boron-doped bioactive glass materials and fluoride on artificially induced root surface caries. Additionally, it was hypothesised that there would be no significant difference in their biocompatibility.

METHODS

Sol-gel synthesis of BG and B_2O_3-doped BG;

Bioactive glasses (BGs) were synthesised according to the literature [22] using a rapid alkali-assisted sol-gel approach. Initially, 20 ml of TEOS was combined with 2.8 ml of 2 M nitric acid and 13.9 ml of distilled water in order to initiate acid-catalysed hydrolysis for 1 hour. Following this process, 2.15 ml of TEP was added, and the mixture was stirred for 20 minutes. Once a clear sol was obtained, 14 g of Ca(NO□)□ was incorporated. Subsequently, 10 ml of 1 M ammonia solution was introduced to induce gelation. The obtained gel was dried at 60 °C for 24 hours, then subjected to heat treatment at 600 °C for 2 hours inside a muffle furnace. In the case of B-doped BG, H□BO□ was incorporated into the system 30 minutes after the TEOS was introduced. The various bioactive glass compositions formulated in this study are presented in Table 1.

Table 1. Bioactive glass compositions

Materials	Reactants (Mole %)			
	SiO□	CaO	P□O□	B□O□
Experimental Bioactive glass	58	38	4	0
Experimental Bioactive glass doped with 7% Boron	51	38	4	7
Experimental Bioactive glass doped with 14% Boron	44	38	4	14

Structural and morphological characterisation of nano-bioactive glass

The phase composition and microstructure of the synthesised nano-bioactive glass were characterised by X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FT-IR) and field emission scanning electron microscopy (FE-SEM), respectively.

XRD analysis of the samples was conducted through a Malvern PANalytical Empyrean MultiCore wide-angle diffractometer equipped with a Cu-Kα radiation source, operated at 40 kV and 40 mA. Measurements were carried out at room temperature over a 2θ range of 20° to 65°, with a scanning rate of 2° per minute. FT-IR spectra were obtained in transmission mode using a Bruker Alpha spectrometer equipped with an attenuated total reflectance (ATR) accessory. The measurements were performed in the wave number range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1}. The surface morphology of the nano-bioactive glass was characterised utilising a Thermo Scientific Apreo 2S FE-SEM.

Sample Preparation

Following the ethical approval, informed consent was obtained from all patients who presented to the Department of Oral and Maxillofacial Surgery at Bezmialem Vakif University Oral and Dental Health Application Centre for the use of their extracted teeth. A total of forty buccal root blocks (n=10 per group), free of carious lesions, were selected from human molars extracted within the past six months for periodontal or orthodontic reasons. Following extraction, the teeth were stored in distilled water until further processing to prevent dehydration and maintain tissue integrity. Sample size estimation was performed through G*Power software (version 3.1.9.7) with a significance level of $\alpha = 0.05$ and a power of 95% ($1-\beta = 0.95$). Based on a previous study, the required number of specimens was determined to be 10 per group [23]. After the cleansing with a fluoride-free prophylactic paste, the extracted teeth were stored in distilled water to prevent dehydration. Each specimen was sectioned under

water 1 mm coronal to the cementoenamel junction, using a precision saw. Subsequently, the obtained samples were examined under a stereomicroscope (Leica MZ7.5, Germany) at 20× magnification to ensure the absence of structural anomalies. Root surfaces were exposed by embedding the samples in acrylic resin. The standardisation of the surfaces was achieved by sequentially polishing using silicon carbide abrasive papers of 600 to 1200 grit. The specimens were then thoroughly rinsed with distilled water.

Artificial demineralisation

Forty samples were immersed in a demineralisation solution (Table 2) for 15 days to induce artificial subsurface lesions. Lesion progression was monitored through a DIAGNOdent Pen (DDP) device (KaVo, Bieberach, Germany). According to the manufacturer's guidelines, readings within the range of 14–20 refer to the initial stages of demineralisation. Only the specimens that exhibited values within this specified range were included in the study.

pH cycle and treatment procedures

The demineralised root samples were randomly distributed to four experimental groups (n=10 per group) as indicated below:

Group F: Fluoride varnish (Duraphat®, Colgate-Palmolive, Germany);

Group BG: Experimental bioactive glass;

Group BG-7: Experimental bioactive glass doped with 7% boron;

Group BG-14: Experimental bioactive glass doped with 14% boron.

Fluoride varnish was applied to the root surfaces with a micro brush per the instructions provided by the manufacturer and left for 4 minutes. For the experimental groups containing bioactive glass and boron-doped bioactive glass powders, a gel-like consistency was achieved by mixing the powders with a phosphate buffer solution (Table 2). The powder-to-liquid ratio

was standardised at 140 mg/300 μL. The prepared mixture was immediately applied to the root surfaces and left for 4 minutes before being rinsed off with distilled water.

A specific pH-cycling regimen was implemented, consisting of a 4-minute application of the test agent, followed by immersion in an acidic buffer solution (Table 2) for 30 minutes. The samples were then rinsed with deionised water for 5 seconds. Subsequently, they were immersed in a neutral buffer solution (Table 2) for 10 minutes, followed by another 5-second rinse with deionised water. This cycle was repeated six times for eight days. Between cycles, the samples were stored in deionised water at 37□°C overnight (Figure 1) [24]. For the *in vitro* pH-cycling experiments, 250 mL polystyrene jars were used, and all solutions were freshly prepared prior to use.

Table 2. Contents of Experimental Solutions and pH

Solutions	Contents	pH
Demineralisation	2.2 mM CaCl□·2H□O, 2.2 mM KH□PO□, 50 mM Acetate	4.6
Phosphate Buffer	137 mM NaCl, 2.7 mM KCl, 10 mM Na□HPO□, 1.8 mM KH□PO□	7.4
Acidic Buffer	50 mM Acetate, 2.25 mM CaCl□·2H□O, 1.35 mM KH□PO□, 130 mM KCl	5.0
Neutral Buffer	20 mM HEPES, 2.25 mM CaCl□·2H□O, 1.35 mM KH□PO□, 130 mM KCl	7.0

Abbreviations: CaCl□·2H□O: Calcium chloride dihydrate, KH□PO□: Potassium dihydrogen phosphate, NaCl: Sodium chloride, KCl: Potassium chloride, Na□HPO□: Disodium hydrogen phosphate, HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

Measurements

The samples were evaluated at three time points: baseline, after demineralisation, and after the treatment procedures.

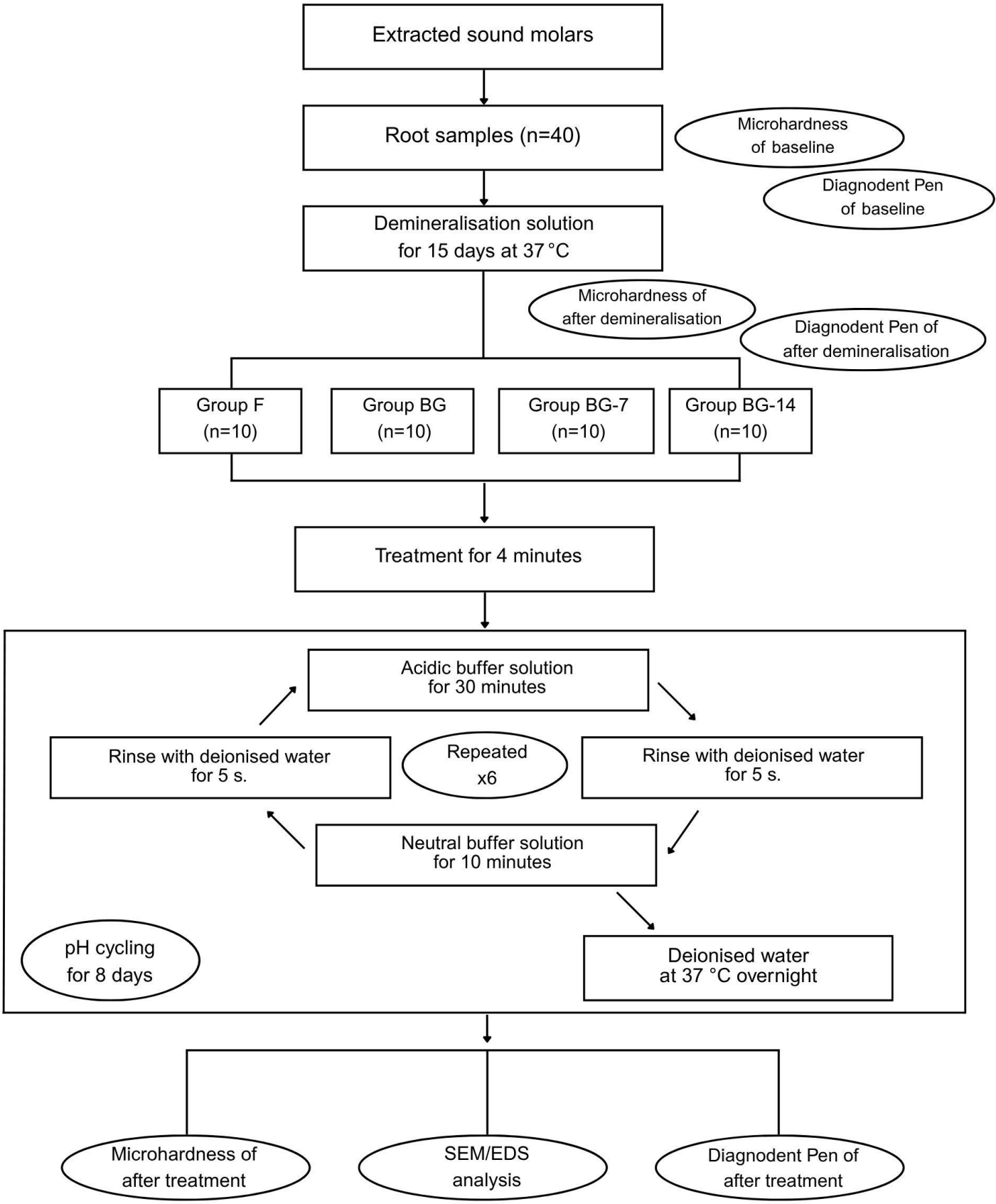

Figure 1. Flowchart of experimental design

Assessment of surface microhardness

Surface microhardness was measured through a microhardness tester (HMV-G, Shimadzu, Japan) equipped with a Vickers elongated diamond indenter and a ×40 magnification objective lens. All microhardness testing procedures were conducted in accordance with the ASTM E92-17 standard. The parameters for the applied load and dwell time were selected within the specified limits of the standard. A load of 980 g was chosen to facilitate the identification and measurement of indentations using the light microscope integrated into the microhardness testing device. A dwell time of 20 s was selected to allow sufficient elastic recovery of the dentine specimens [25]. All microhardness measurements were performed by a single operator (R.F.S.). Three different points were measured on each sample, and the obtained mean value was recorded to conduct the analysis. Employing a precision microscope integrated with microhardness testing, this method is an example of the most widely used techniques in *in vitro* studies for assessing remineralisation. The indentations were observed under ×400 magnification, and their diagonal lengths were measured using the device's built-in scale microscope. Following the process, the surface microhardness values were obtained through conversion from the Vickers Hardness Numbers (VHN).

Assessment of fluorescence properties: DIAGNOdent Pen Devices

The DDP (KaVo, Bieberach, Germany) was calibrated prior to each measurement session in line with the instructions of the manufacturer, using a calibration disc, and a zero baseline was produced using a sound spot. The conical tip (probe 1) of the DDPen was placed perpendicular to the sample's surface, and its vertical axis was adjusted to obtain the maximum reading. Three measurements were obtained from each sample, and the resulting DDP values were recorded for analysis. All DDP measurements were performed by a single operator (R.F.S.).

Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy analyses

One representative sample from each group was subjected to surface characterisation using Scanning Electron Microscopy combined with Energy Dispersive X-ray Spectrometry (SEM-EDS) (Evo ls10, Zeiss, Germany). The analysis was performed at an acceleration voltage of 10 kV and a magnification of x1000, x3000 and x5000. The focus of the evaluation was on the central region of the root surface, with specific attention to the detection and distribution of the oxygen (O), phosphorus (P), calcium (Ca), fluoride (F), silicon (Si), magnesium (Mg), aluminium (Al), and boron (B) elements. Prior to the SEM-EDS analysis, sputter-coating with a thin layer of gold-palladium mixture was applied to all samples to enhance conductivity. The atomic percentages of the detected elements were recorded.

Cell viability assay

Human dermal fibroblast cells (CCD-1072Sk) were seeded into 96-well plates at a density of 5,000–10,000 cells per well in 100 µL of complete growth medium and incubated at 37 °C in a humidified atmosphere containing 5% CO for 24 hours to allow cell adherence. The existing culture media were replaced with media that had been previously incubated with the bioactive materials and fluoride for 24 hours, allowing the release of their ionic and molecular components into the medium. Then, they were maintained under standard culture conditions for 24 and 48 hours. Subsequently, 10 µL of WST-1 reagent (Cayman Chemical, USA) was added directly to each well, and the plates were further incubated for 4 hours at 37 °C. The assessment for cell viability was then conducted by measuring absorbance at 450 nm through a microplate reader (Varioskan, Thermo, USA). Absorbance values were normalised to those of the untreated control group after subtracting the background signal obtained from wells containing medium and WST-1 without cells.

Statistical analysis

Statistical analyses were conducted through IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY, USA). Mean surface microhardness and laser fluorescence values were calculated for each group. A significant level of $p < 0.05$ was employed for all tests. For each sample, three surface microhardness measurements—baseline, after demineralisation, and after treatment— along with DDP readings were recorded. Intra-group and inter-group comparisons were carried out through repeated measures analysis of variance (ANOVA), and post hoc pairwise comparisons were conducted through the Bonferroni correction.

For biocompatibility assessment, cell viability assays were analysed. A one-sample t-test with Bonferroni correction was applied for comparisons between the control (100%) and experimental groups within each material group, with $p < 0.0125$ considered statistically significant. For comparisons among groups within each dose, ANOVA was performed, followed by Tukey's post hoc test for pairwise comparisons ($p < 0.05$).

RESULTS

Physicochemical characterisation studies

FT-IR spectra (Figure 2a) were used to characterise BG and boron-doped BG materials. From FT-IR spectra of BG and boron-doped BG materials, characteristic peaks attributed to asymmetric stretching of Si-O-Si forming the structure of bioglass are seen around 1000-1100 cm^{-1}. From the observations, it can be said that the peak intensity decreases as boron doping increases on bioglass. The absorption peak observed at 1300-1350 cm^{-1} indicates the B-O stretching vibrations of BO_3 units. In addition, the presence of absorption bands at 1740 cm^{-1} in the FT-IR spectra of all samples confirmed the hygroscopic structure of silicate glass, and this absorption band occurs due to the bending vibrations of O-H groups. In addition, the absorption band regarding the carbonate group was recorded around 1200 cm^{-1}.

To evaluate the crystalline/amorphous structure of BG, the XRD patterns of BG and boron-doped BGs were analysed (Figure 2b). The BG pattern only shows a peak at 2θ=29.40°, which is attributed to calcium silicate (Ca_2SiO_4) [22,26]. The XRD patterns of all boron-doped BGs point out an increase in the widening range of the main diffraction peak of BG, which confirms their amorphous nature. Also, according to literature data [27], the XRD of B_2O_3 is seen around 2θ=29°. Based on the literature, the broadening of the main diffraction peak of boron-doped BG can also be attributed to the increase in boron doping.

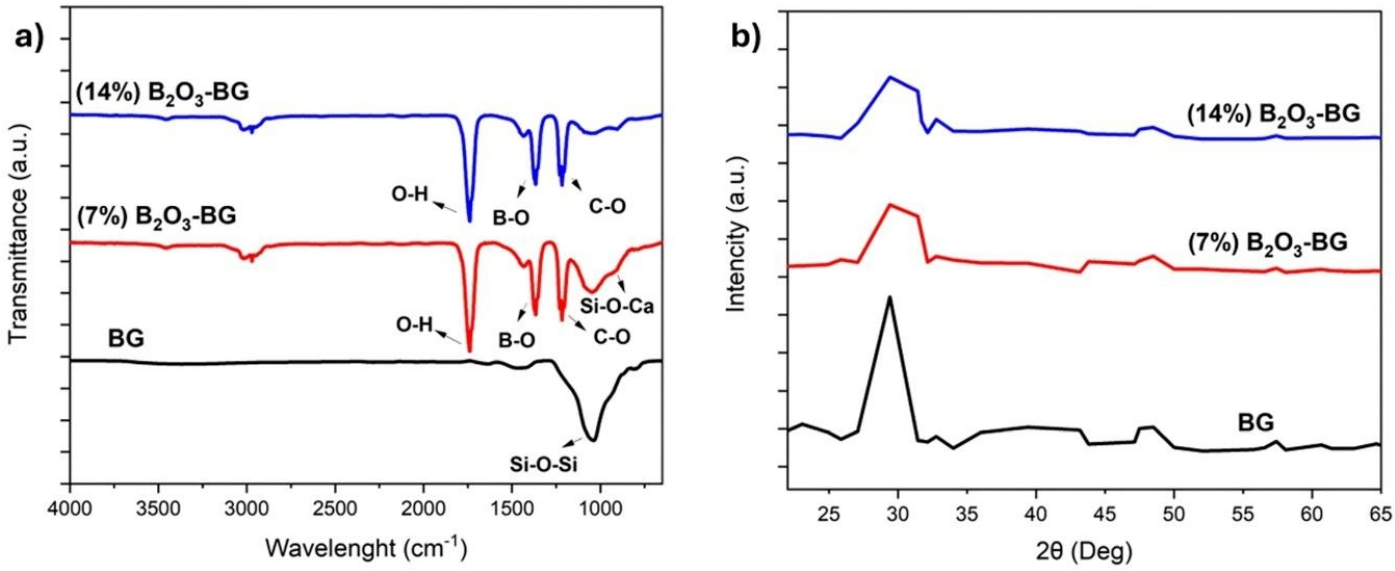

Figure 2. a) FT-IR spectra of BG and boron-doped BG materials, calcined at 600 °C; b) XRD patterns of BG and boron-doped BG materials, calcined at 600 °C.

The pattern observed in the FE-SEM image of bioactive glass is also observed in boron-doped bioactive glass powders (Figure 3). The FE-SEM images revealed an irregular, porous surface structure, which is characteristic of bioactive glasses designed to facilitate ion exchange and cell attachment. The presence of interconnected porosities suggests a high surface area, which is beneficial for bioactivity and potential remineralisation capability. No signs of phase separation or crystalline structures were observed, indicating that the material maintained its

amorphous nature following synthesis. The homogeneity of the surface further supports the successful incorporation of boron into the glass matrix.

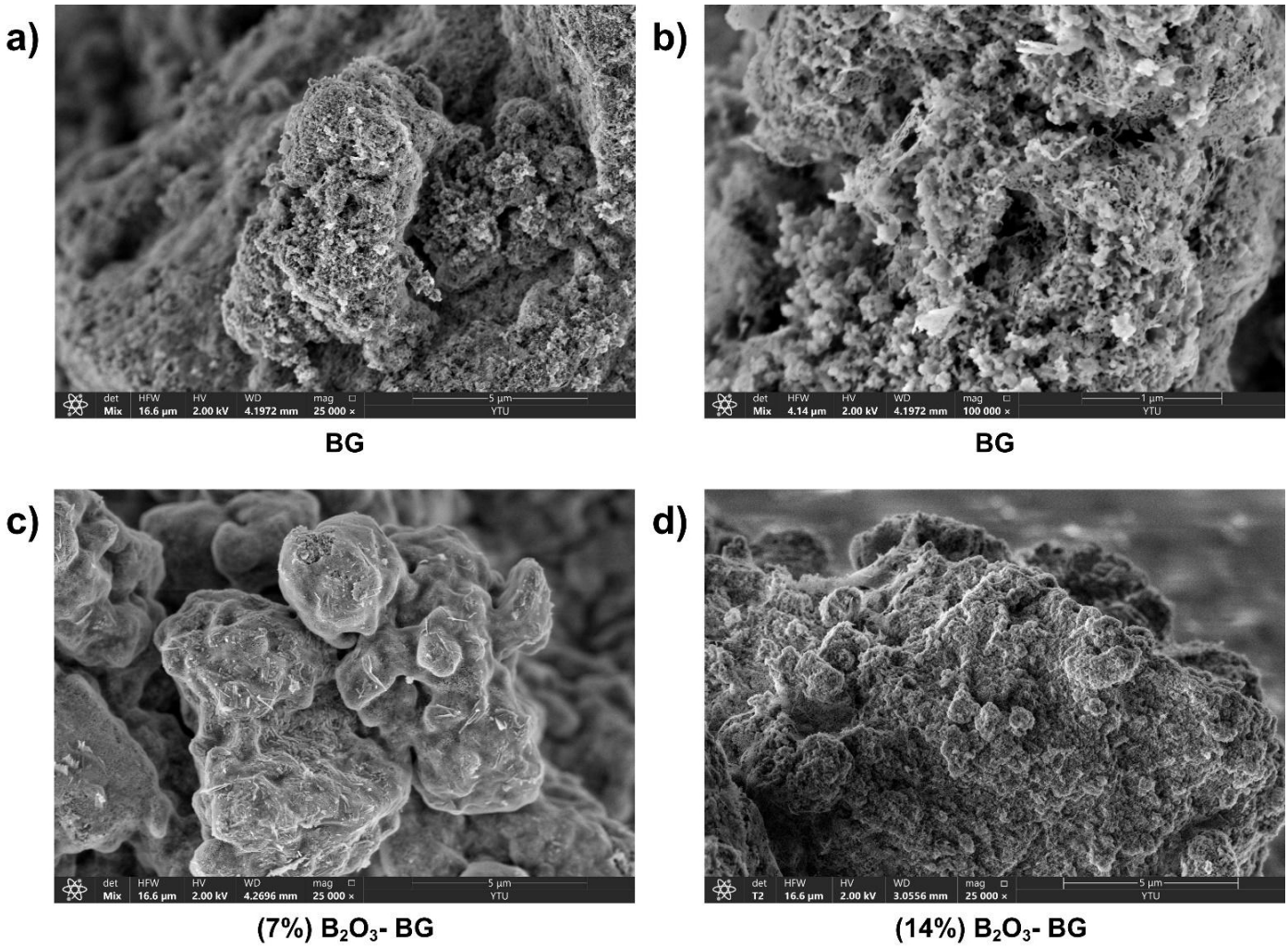

Figure 3. FE-SEM images of a-b) BG, c) (7%) B_2O_3- BG, d) (14%) B_2O_3- BG

Microhardness Results

The microhardness values obtained from the experimental groups and their statistical comparisons are demonstrated in Table 3. No significant differences were obtained among the baseline microhardness values ($p > 0.05$). Following demineralisation, a significant decrease in microhardness was detected in all groups ($p < 0.05$). After remineralisation treatment, microhardness values significantly increased across all groups. After treatment, microhardness values were found to be comparable to baseline measurements ($p > 0.05$). No statistically

significant differences were examined among the treatment groups following remineralisation ($p > 0.05$).

Table 3. Microhardness results

	Baseline	After Demineralisation	After treatment	p
Group F	59.45±4.72[b]	25.43±1.54[a]	64.58±4.56[b]	<0.001
Group BG	61.15±6.12[b]	25.38±1.87[a]	61.50±5.81[b]	<0.001
Group BG-7	60.58±4.35[b]	24.30±1.52[a]	65.72±5.12[b]	<0.001
Group BG-14	60.57±4.05[b]	24.19±1.85[a]	63.47±4.07[b]	<0.001
P	0.884	0.213	0.261	

Different lowercase letters within the same row indicate statistically significant differences between groups ($p < 0.05$).

DIAGNOdent Pen Results

The DDP values of the groups and their statistical comparisons are demonstrated in Table 4. No statistically significant differences were examined among the baseline measurements of the groups ($p > 0.05$). Following demineralisation, it was observed that DDP scores increased in all groups. After treatment, a decrease in these scores was observed across all groups. Among the post-treatment measurements, the lowest DDP score was found in Group BG-14 ($p < 0.05$). No significant differences were identified among the other treatment groups ($p > 0.05$).

Table 4. DIAGNOdent Pen Results

	Baseline	After Demineralisation	After treatment	p
Group F	5.200±0.613[a]	14.967±1.494[c]	7.600±1.616[bB]	<0.001
Group BG	5.152±0.821[a]	14.515±1.004[c]	8.333±1.308[bB]	<0.001
Group BG-7	4.967±0.675[a]	15.700±1.535[c]	9.267±1.698[bB]	<0.001
Group BG-14	4.733±0.940[a]	14.333±1.324[b]	5.167±0.741[aA]	<0.001
p	0.525	0.122	<0.001	

Different lowercase letters in the same row and different uppercase letters in the same column indicate statistically significant differences ($p < 0.05$).

Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy Analysis Results

Representative SEM images of all groups are provided in Figure 4a and Figure 4b**.** In the Baseline image, an intact dentine surface was observed. After demineralisation, the dentinal tubules appeared noticeably widened and exposed. In the fluoride-treated sample, the dentinal tubules were occluded with dense deposits, exhibiting a concrete-like appearance. In contrast, in the group treated with experimental bioactive glass, most of the dentinal tubules remained open. Nevertheless, in the groups treated with bioactive glass containing 7% and 14% boron, a tubule occlusion pattern similar to that of the fluoride group was observed.

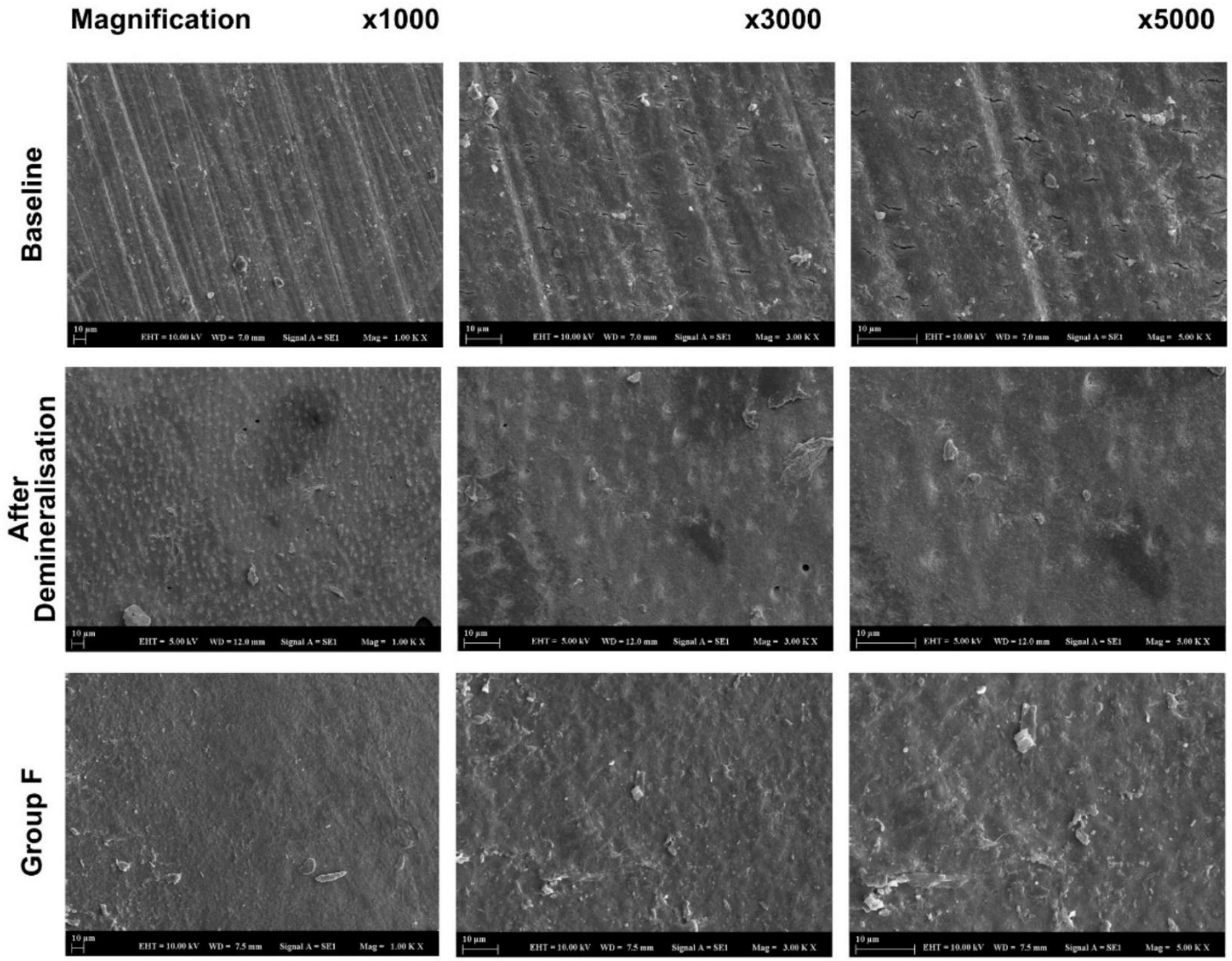

Figure 4a. SEM images for baseline, after demineralisation and Group F at x1000, x3000 and x5000 magnifications.

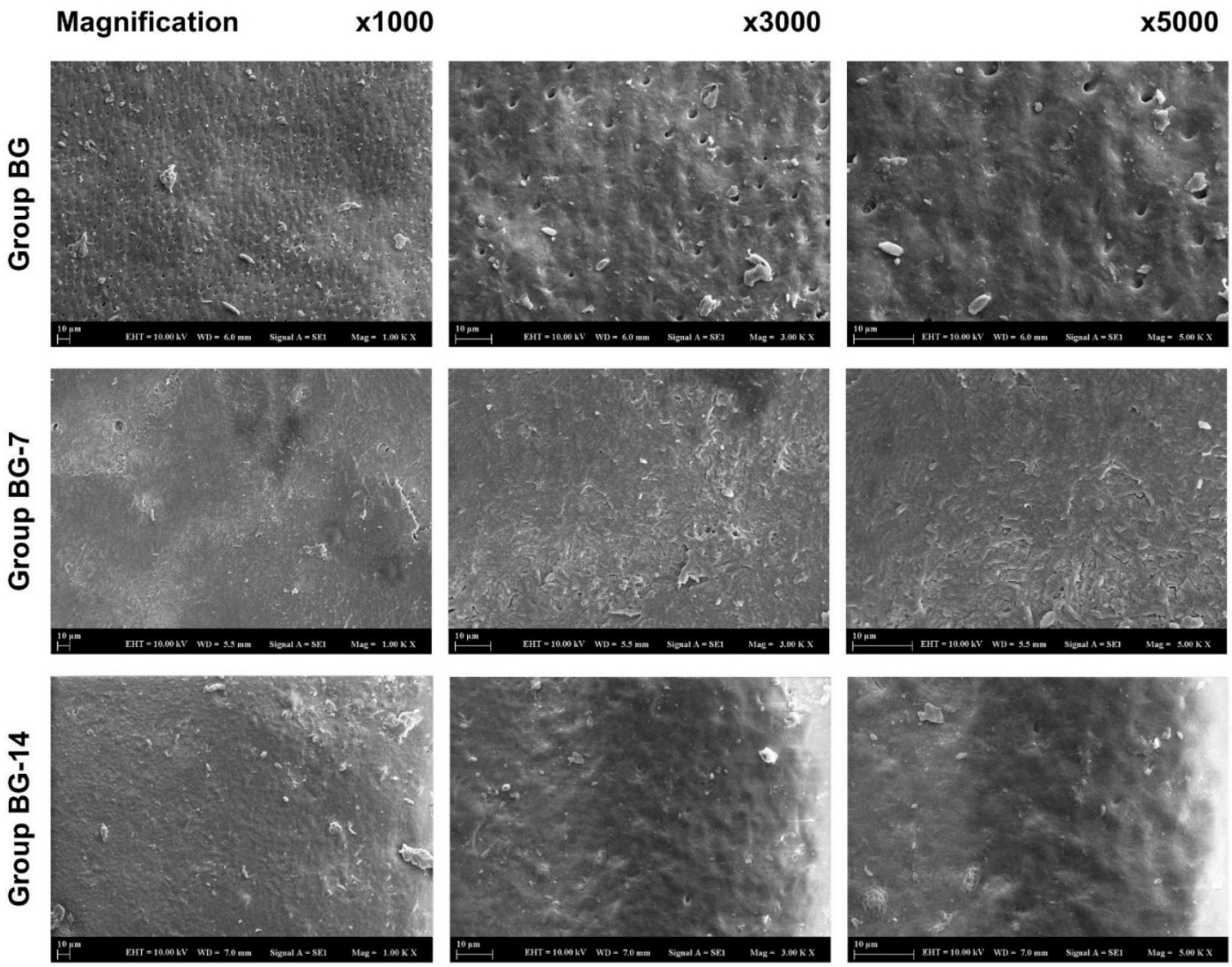

Figure 4b. SEM images for Group BG, Group BG-7 and Group BG-14 at x1000, x3000 and x5000 magnifications.

The representative semi-quantitative chemical analyses and element distribution for all tested groups are given in Figure 5. The EDS analysis revealed significant variations in surface elemental composition among the treatment groups (Table 5). The Baseline group showed high Ca and P levels, which decreased after demineralisation. The fluoride-treated positive control group (Group F) partially preserved Ca and P and contained detectable F. Bioactive glass-treated groups exhibited elevated F, B, and Si levels, with Group BG-14 showing a notably high B content (5.64%). Increased Ca and P in BG-7 and BG-14 indicated enhanced remineralisation. Overall, bioactive glass treatments positively modified surface mineral composition, with all

treated groups showing profiles comparable to or better than the positive control. The Ca/P ratio obtained in the Baseline group (1.24) reflected the natural mineral content of dentine tissue, while the decrease of this ratio to 0.29 in the after demineralisation clearly indicated surface demineralisation. In the groups treated with fluoride (Group F) and bioactive glass (Group BG), the Ca/P ratios remained low at 0.21 and 0.20, respectively, suggesting that these treatments did not significantly alter the surface mineral profile and that new mineral deposition was limited. In contrast, the increase in the Ca/P ratio to 1.28 in Group BG-7 indicated more effective mineral accumulation on the surface during the remineralisation process and demonstrated the enhanced remineralisation potential of boron-doped bioactive glass over time.

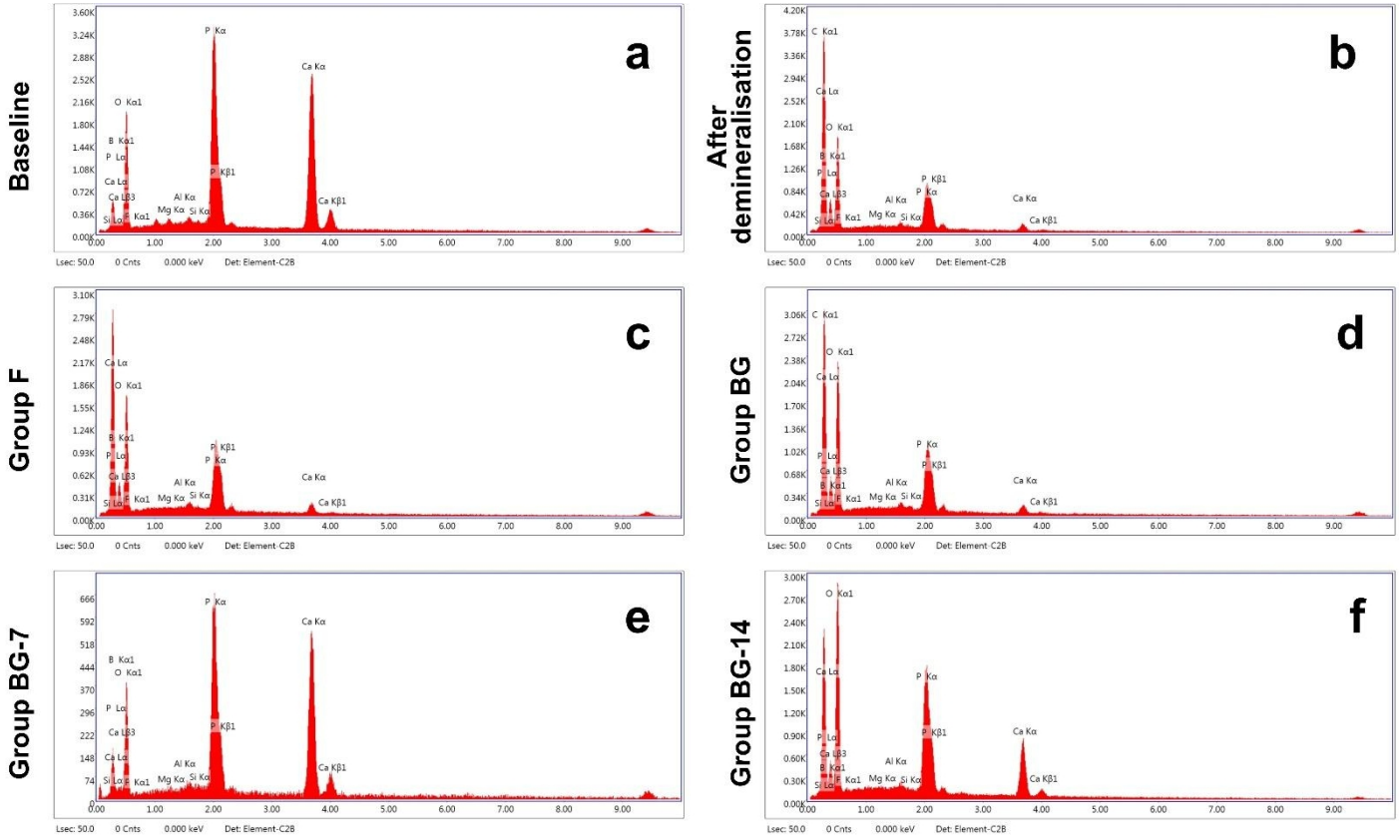

Figure 5. Semi-quantitative chemical analysis and distribution of elements. a. Baseline, b. After demineralisation, c. Group F, d. Group BG, e. Group BG-7, f. Group BG-14.

Table 5. Elemental composition (atomic%) and Ca/P ratio for representative samples from each group using EDS analysis

Element	Baseline	After demineralisation	Group F	Group BG	Group BG-7	Group BG-14
B	0.03	0.01	0.03	0.02	0.16	5.64
O	51.47	32.12	60.68	56.85	49.98	64.16
F	0.19	0.33	0.23	35.2	0.41	1.78
Mg	0.24	0.39	0.27	0.41	0.12	1.09
Al	0.12	0.39	0.79	0.32	0.22	0.68
Si	0.11	0.45	0.5	0.34	0.37	0.39
P	21.34	10.02	19.53	5.45	21.34	16.34
Ca	26.49	2.91	4.19	1.1	27.39	9.93
Ca/P	1.24	0.29	0.21	0.20	1.28	0.61

Abbreviations: B: Boron, O: Oxygen, F: Fluorine, Mg: Magnesium, Al: Aluminium, Si: Silicon, P: Phosphorus, Ca: Calcium

Cell viability results

In the viability assays, the cytotoxic effects of dental materials at different concentrations on fibroblast cells were evaluated. The results revealed that the material exhibiting the highest level of cytotoxicity on the CCD-1072Sk at both 24-hour and 48-hour time points was fluoride-containing material when compared to the control group (untreated cells). Notably, within the first 24 hours of exposure, approximately 50% cell death was observed even at the lowest concentration of the fluoride-containing material (Table 6, Figure 6). Nevertheless, at the 48-hour time point, the cytotoxic effect appeared to decrease when compared to the control group, suggesting a reduction in the material's toxic impact over time (Table 7, Figure 7). These findings indicate that the cytotoxic effect of fluoride was more prominent during the initial 24-hour period.

When evaluating the bioactive materials, all three bioactive glass-based groups (BG, BG-7, and BG-14) exhibited considerably higher levels of cell viability across the tested concentrations in 24-hour exposure (Table 6, Figure 6). Group BG-7 showed the most favourable results, maintaining viability above 80% even at the highest dose, with statistically significant cytotoxicity observed only at higher concentrations. Group BG also demonstrated similar

biocompatibility, with viability values remaining close to the control group at lower concentrations and only slightly decreasing at higher doses. Group BG-14 followed a comparable pattern at lower concentrations but showed an evident decline in cell viability at 5 mg/mL, suggesting that concentration may contribute to increased cytotoxicity at higher exposure levels.

In the viability of the 48-hour exposure group, Group BG maintained relatively higher viability, although a dose-dependent decrease was observed—from 106.49% ± 4.47 at the lowest concentration to 52.82% ± 1.71 at the highest, with statistical significance observed at medium to high concentrations ($p = 0.003$). Notably, Group BG-7 and BG-14 showed the highest levels of cell proliferation, particularly at the lower concentrations. Group BG-7 reached up to 169.03% ± 2.57 at the lowest dose, while Group BG-14 reached 170.66% ± 6.92, both significantly higher when compared to the control group ($p < 0.001$ and $p = 0.003$, respectively). Although viability declined at higher concentrations, especially for BG-14, values remained above or near 70% even at the highest dose. These findings suggest that bioactive glass materials — particularly BG-7 and BG-14— may not only be less cytotoxic but could potentially promote fibroblast proliferation at lower concentrations. Overall, these results highlight the superior cytocompatibility of bioactive glass materials compared to fluoride, particularly in short-term exposure scenarios.

Table 6. Cell proliferation in CCD-1072 sk cell line at 24-hour treatment with test materials

					p (compare with control-100)			
	0.63 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL	0.63 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL
Group F	58.74±3.43[a]	41.42±7.21[a]	25.28±4.50[a]	17.75±0.42[a]	**0.002**	**0.005**	**0.001**	**<0.001**
Group BG	105.82±3.56[c]	85.11±3.84[b]	83.97±1.46[b]	80.45±0.85[c]	0.105	0.021	**0.003**	**0.001**
Group BG-7	97.76±1.85[bc]	88.8±4.38[b]	87.83±2.44[b]	81.59±1.65[c]	0.170	0.047	0.013	**0.003**
Group BG-14	91.39±6.36[b]	87.91±7.55[b]	82.3±1.69[b]	57.85±0.94[b]	0.144	0.109	**0.003**	**<0.001**
p	<0.001	<0.001	<0.001	<0.001				

There are significant differences between the groups represented by different lowercase letters within the same column. Bonferroni correction was applied for comparisons made with the control group (i.e., with the value 100) within each material group, and a p-value of <0.0125 was considered statistically significant (significant comparisons are indicated in bold).

Table 7. Cell proliferation in CCD-1072 sk cell line at 48-hour treatment with test materials

					p (compare with control-100)			
	0.63 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL	0.63 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL
Group F	48.35±1.12[a]	42.28±1.01[a]	41.61±0.73[a]	42.84±2.82[a]	**<0.001**	**<0.001**	**<0.001**	**0.001**
Group BG	106.49±4.47[b]	85.8±5.19[b]	57.39±3.07[b]	52.82±1.71[b]	0.128	0.042	0.013	**0.003**
Group BG-7	169.03±2.57[c]	155.87±7.07[c]	101.91±6.83[c]	57.39±3.07[b]	**<0.001**	**0.005**	0.676	**0.002**
Group BG-14	170.66±6.92[c]	173.37±3.85[d]	97.24±1.59[c]	71.6±6.13[c]	**0.003**	**0.001**	0.095	0.015
p	<0.001	<0.001	<0.001	<0.001				

There are significant differences between the groups represented by different lowercase letters within the same column. Bonferroni correction was applied for comparisons made with the control group (i.e., with the value 100) within each material group, and a p-value of <0.0125 was considered statistically significant (significant comparisons are indicated in bold).

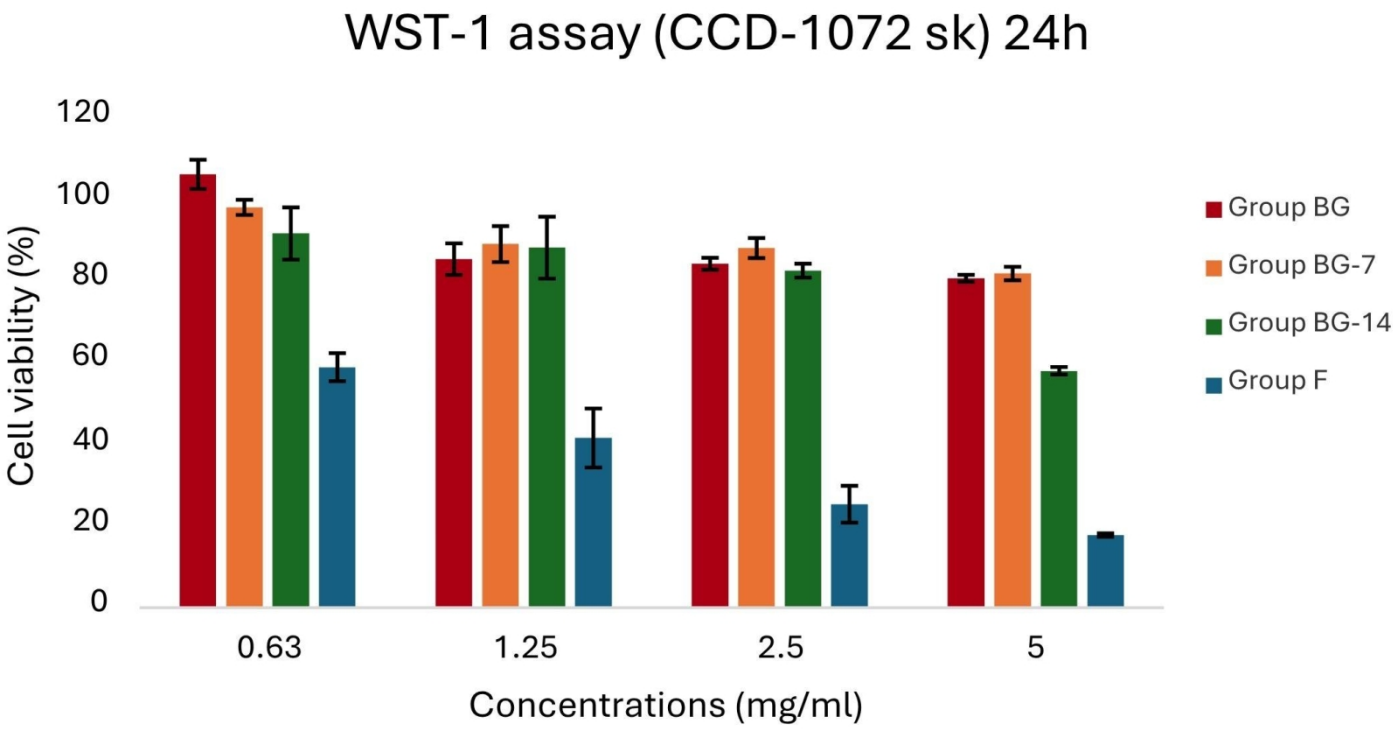

Figure 6. 24-hour cell viability (%) of all test materials

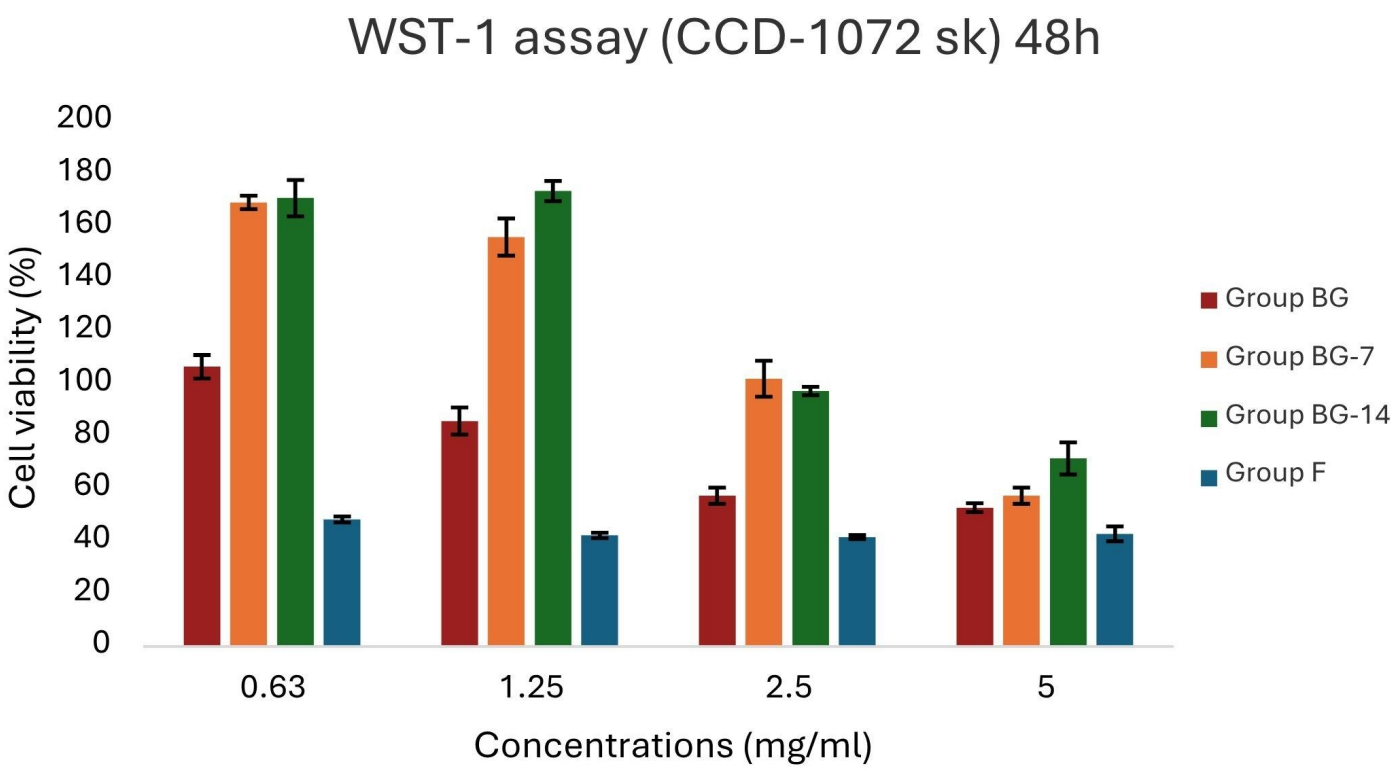

Figure 7. 48-hour cell viability (%) of all test materials

DISCUSSION

This research aimed to evaluate the remineralisation effects and biocompatibility of boron-doped bioactive glass materials on artificially induced root surface caries. According to the obtained data, no significant differences were monitored among the tested groups regarding microhardness results after treatment; nonetheless, DDP assessment revealed a significant difference in the group treated with bioactive glass containing 14% boron compared to the other groups. According to the SEM-EDS analysis results, the increase in the Ca/P ratio to 1.28 in the BG-7 group indicated more effective mineral accumulation on the surface during the remineralisation process. Regarding cellular cytotoxicity, fibroblast viability was maintained in the boron-doped bioactive glass groups, whereas cytotoxic effects were observed with fluoride. The null hypothesis of this research was that there would be no significant difference in the remineralisation effects between boron-doped bioactive glass materials and fluoride on artificially induced root surface caries. Additionally, it was hypothesised that there would be no

significant difference in their biocompatibility. Based on the results of this study, the null hypothesis suggesting that there would be no significant difference in remineralisation efficacy and biocompatibility between boron-doped bioactive glass materials and fluoride is therefore rejected.

The findings of this study are particularly relevant in the context of geriatric dentistry, where early intervention and conservative treatment approaches are favoured due to the challenges elderly individuals may face in maintaining complex restorative treatment [28]. Root caries is highly prevalent in older adults, highlighting the importance of developing and implementing preventive strategies based on minimally invasive treatments tailored to individual needs [29].

Modern concepts in minimally invasive dentistry emphasise the importance of arresting and reversing early lesions through the use of topical remineralising agents [30]. Fluoride is the most commonly used agent in dental remineralisation and is considered effective in arresting caries progression in both enamel and dentine tissues. On root surfaces in particular, fluoride ions interact with hydroxyapatite crystals in dentine to promote the formation of fluorapatite, thereby enhancing resistance to demineralisation [31]. Nevertheless, the remineralisation effect of fluoride is largely limited to the surface, with insufficient ion diffusion into deeper lesion areas [32]. Additionally, the potential risk of cytotoxicity should be considered, especially when fluoride is used in high concentrations or applied frequently. Although fluoride remains a well-established agent for enamel remineralisation, its limited efficacy on dentine—attributed to the lower mineral content and the presence of a collagen-rich organic matrix susceptible to enzymatic degradation—necessitates alternative strategies [33-35].

In this context, the development and the evaluation of novel materials such as boron-doped bioactive glass become increasingly important. These materials not only aim to promote effective remineralisation but also support the preservation of dentine's organic structure,

offering a promising approach to slow down the progression of root caries, especially in elderly populations [36].

In vitro pH-cycling models aim to replicate the fluctuations in mineral saturation and pH that occur during the natural progression of dental caries [37]. The pH-cycling procedure used in this study was applied to mimic the oral environment by subjecting artificial carious lesions to repeated acid challenges followed by remineralisation.

In this study, both the microhardness testing and the DDP device were utilised to evaluate remineralisation. Although both methods have been widely employed in the literature to monitor demineralisation and remineralisation processes in enamel tissue, studies that combine these two methods specifically on root surfaces (dentine tissue) are quite limited. Therefore, this study aims to fill a significant gap in the literature. The microhardness testing has been extensively applied in numerous *in vitro* studies to assess the remineralisation potential of various agents. In the present study, the VHN test was selected due to its advantages of being a rapid, reliable, economical, and non-destructive method [38]. The DDP is also a frequently preferred method because it is easy to apply, reproducible, fast, and has demonstrated reliability in both *in vivo* and *in vitro* studies. On the other hand, when compared to enamel, dentine has a lower mineral content and a more complex tubular structure, which may reduce the sensitivity of fluorescence-based measurements performed with the DDP device [39].

In this study, the effects of different remineralisation agents on artificially induced root surface caries were evaluated. According to the obtained data, no significant differences were observed between baseline and after-treatment measurements within any of the groups. This finding suggests that the applied treatments have the potential to reverse demineralisation on root surfaces. Furthermore, no statistically significant differences were found between the groups related to their remineralisation efficacy. Fluoride application is currently the most commonly employed method for preventing dental caries through remineralisation. Its effectiveness in

remineralisation therapies has been extensively documented in numerous studies, establishing it as the gold standard. According to the microhardness test results, the remineralisation effect observed in the tested groups was comparable to that of fluoride [9]. Longer application times of the test material under *in vitro* conditions may enhance mineral deposition on the surface, thereby leading to an increased remineralisation effect. In addition to that, no statistically significant differences were determined between bioactive glass groups containing 7% and 14% boron.

Upon evaluation of the DDP scores, no significant difference was detected between the baseline and final measurements in the group treated with 14% boron-doped bioactive glass. This suggests that the material may be effective in suppressing caries progression. Although higher scores were recorded in the other groups (Group F, Group BG, and Group BG-7) compared to their baseline values, these increases were not statistically significant among the groups. While remineralisation was achieved in these groups as well, the scores did not reach the level of sound root surfaces. These findings indicate that the contribution of 14% boron-doped glass to the remineralisation process may be comparable to that of healthy root surfaces. In a study evaluating enamel remineralisation using 58S5 bioactive glass and fluoride varnish, both microhardness testing and DDP were used, and results similar to those of this study were reported [40].

Upon examination of the SEM images, the initially root surface dentine structure was distinctly replaced by exposed dentinal tubules following the demineralisation process. After treatment, the fluoride group exhibited occlusion of the open tubules and a smooth surface topography. Although complete occlusion comparable to fluoride was not observed in the bioactive glass group, narrowing of the tubules was evident. In the boron-containing groups, a surface morphology similar to that of the fluoride group was observed. These findings may suggest that the incorporation of boron enhances the stability of bioactive glass materials on root surfaces.

In a study evaluating the remineralisation effect of strontium-doped bioactive glass on dentine surfaces, findings consistent with the present study revealed that the use of all three BG compositions resulted in the occlusion of exposed dentinal tubules, as evidenced by SEM imaging [41].

The EDS analysis results demonstrated that the different treatment protocols applied in this study had significant effects on the elemental composition of the surface. The high levels of Ca and P observed in the baseline group reflect the inherent mineral content of natural enamel, whereas the decrease of these elements in the after-demineralisation group suggests mineral loss or transient alterations in the surface structure. In the fluoride-containing positive control group (Group F), the partial preservation of Ca and P levels, along with the detection of F on the surface, indicates that fluoride treatment supports remineralisation and stabilises the enamel structure. In the bioactive glass-containing groups, high levels of F, B, and Si were detected on the surface. The increase in Ca and P levels further indicates the progression of remineralisation. These findings support the role of bioactive glass treatment in improving and reinforcing surface chemistry, with boron playing a potentially important role in remineralisation mechanisms. Overall, the changes in elemental composition demonstrate that the treatment groups exhibited mineralisation profiles comparable to or superior to the positive control group, providing important evidence that the applied treatments effectively promote remineralisation. This study determined elemental percentages by obtaining a single sample from each group; nonetheless, a more extensive and detailed analysis is necessary for more reliable results.

In this study, the CCD fibroblast cell line was used for cell viability assessment. The primary rationale for this choice was its high proliferative capacity, ease of culture, and reproducibility of results *in vitro***,** which make it a widely accepted standard model in biomaterials research. CCD fibroblasts are well-characterised, commercially available, and provide consistent responses, allowing for reliable comparison between experimental groups [42].

The cell viability results suggest that fluoride may exert cytotoxic effects on fibroblast cells *in vitro*, particularly during the initial 24 hours of exposure, with the effect appearing to decrease over time. This finding is consistent with a previously conducted study [43], in which the metabolic activity of hGFs exposed to different concentrations of desensitising varnishes was evaluated through MTT assays. In that study, Duraphat varnish at a low concentration (0.1%) demonstrated similar cell viability to the control group. Moreover, consistent with our results, cytotoxicity decreased after 48 hours of incubation compared to 24 hours, while higher concentrations led to increased cytotoxic effects. In contrast, it contradicts another study, which reported that sterilised root surfaces did not exhibit cytotoxic effects *in vitro*, even after additional treatment with fluoride or fluoride plus citric acid [44]. In that study, fluoride was not applied directly to the fibroblast cells; instead, it was applied to the root surfaces before incubation with human gingival fibroblasts (hGFs). These discrepancies may be due to differences in fluoride concentrations used, methods of application, and variations in the cell types or culture conditions employed.

In a study evaluating the cytotoxicity of three different bioactive glass formulations under *in vitro* conditions on various cell types and species, it was reported that bioactive glass was not cytotoxic to human fibroblast cells, in line with the findings of this study [45]. In a study evaluating the effects of different bioactive glasses on metabolism, intracellular ion concentrations, and cell viability, bioactive glass was reported to be a biocompatible material, which supports the findings of this study [46]. In the same study, under the tested conditions, none of the three bioactive glasses consistently or significantly enhanced cell proliferation or increased alkaline phosphatase (ALP) activity. Similarly, in this study, the experimental bioactive glass did not affect cell proliferation, but the increase observed in cell proliferation in the boron-containing groups was noteworthy. On the other hand, another study demonstrated that boron-rich surface pre-reacted glass-ionomer (S-PRG) filler eluates enhanced ALP activity

while suppressing the proliferation of human dental pulp stem cells (hDPSCs). The primary aim of the study was to clarify the specific role of boron released from S-PRG fillers [47].

In this study, the effects of boron-doped bioactive glass material on the normal epithelial cell line (CCD-1072SK) were investigated to elucidate its potential role in cell viability. The findings of this study suggest that boron may exert modulatory effects on cellular proliferation and viability in epithelial cells at a certain concentration after 48 hours of exposure time. These effects appear to be dose-dependent, with lower concentrations promoting cell viability, while higher concentrations may lead to cytotoxicity. Previous studies have highlighted boron's involvement in various biological processes, including bone metabolism, wound healing, and anti-inflammatory responses [48]. The observed proliferative effect at lower boron concentrations in this study is consistent with literature reporting boron's ability to enhance cell growth and tissue regeneration, particularly in hDPSCs [49]. In another study, boron nitride nanotubes were shown to enhance dental adhesion without compromising the viability of fibroblast cells. Similarly, in this current study, boron-doped bioactive glasses did not result in a significant reduction in cell viability, except at the 5% concentration [50]. Moreover, boron's interaction with key signalling pathways—such as the Wnt/β-catenin pathways—may underlie its regulatory effects on epithelial cell behaviour [51]. While this study did not investigate a pathway directly, the patterns observed in cellular activity imply that boron may influence intracellular signalling relevant to epithelial cell function and integrity. These findings suggest that boron, when used at appropriate concentrations, can support bioactivity without exerting cytotoxic effects on epithelial cells.

This study has several limitations to be considered. The *in vitro* design, although enabling controlled assessment, does not fully replicate the complex biological and environmental conditions of the oral cavity, where saliva, biofilm, and host responses are critical to caries progression and repair. The artificial demineralisation and pH-cycling protocols, while

standardised, cannot fully mimic the dynamic and multifactorial *in vivo* situation. Although the sample size was based on G*Power analysis, variability in dentine substrates may not have been fully captured, raising the possibility of underpowering for subtle intergroup differences. Only two boron doping concentrations were evaluated, which restricts the conclusions regarding dose–response effects. In the SEM-EDS analysis, only one representative sample per group was examined, limiting the reliability and generalisability of the elemental findings. The relatively short duration of the pH-cycling further restricts assessment of long-term remineralisation stability. A further limitation is the use of CCD fibroblasts rather than primary gingival or periodontal fibroblasts which may better reflect the oral environment. While CCD fibroblasts ensure reproducibility, they cannot fully model the biological responses of native oral cells and, therefore, the cytocompatibility results should be interpreted with caution.

Future studies incorporating a broader range of boron concentrations, clinically relevant cell models (such as hDPSCs or gingival fibroblasts), and long-term *in vivo* validation under cariogenic conditions are needed to determine the optimal therapeutic window and establish the true clinical potential of boron-doped bioactive glasses. While the findings of this study indicate surface remineralisation, the actual bonding capability of boron-doped bioactive glasses to dental tissues remains to be clarified. Future studies focusing on adhesion to tooth structure and interfacial characterisation are necessary to address this aspect.

CONCLUSION

This study demonstrated that boron-doped bioactive glasses may enhance remineralisation of root surface caries while maintaining acceptable biocompatibility. A significant increase in microhardness values was observed in all groups following remineralisation, returning to baseline levels, with no statistically significant differences between the groups. DDP analyses confirmed remineralisation in all groups, with the highest reduction in demineralisation signals

detected in the group treated with 14% boron-doped bioactive glass, suggesting a potential dose-dependent effect. The SEM-EDS analyses further supported these findings by showing increased mineral deposition, particularly in the group containing 7% boron, as evidenced by higher Ca/P ratios and favourable elemental distributions. These results were corroborated by the SEM images, where boron-doped bioactive glasses exhibited surface coverage patterns on exposed dentine similar to fluoride-treated samples. Cell viability assays indicated that boron-doped bioactive glasses supported fibroblast proliferation at lower concentrations and showed lower cytotoxicity compared to fluoride.

Nevertheless, considering the limitations of an *in vitro* setting, the extrapolation of these findings to clinical dentistry should be made with caution. In summary, boron-doped bioactive glasses may offer a biocompatible and clinically promising alternative to fluoride in clinical applications. In particular, the optimal boron concentration and the possible long-term effects on dental tissues and surrounding oral structures remain unclear. Future studies involving clinically relevant cell models, varied application protocols, and long-term *in vivo* experiments are required before definitive conclusions can be drawn regarding clinical applicability.

List of abbreviations

NaF: Sodium fluoride

BG: Bioactive glass

XRD: X-ray diffractometry

FT-IR: Fourier transform infrared spectroscopy

ATR: Attenuated total reflectance

FE-SEM: Field emission scanning electron microscopy

VHN: Vickers microhardness numbers

SEM: Scanning electron microscopy

EDS: Energy dispersive x-ray spectroscopy

ANOVA: Analysis of variance

DDP: DIAGNOdent Pen

$CaCl_2 \cdot 2H_2O$: Calcium chloride dihydrate

KH_2PO_4: Potassium dihydrogen phosphate

NaCl: Sodium chloride

KCl: Potassium chloride

Na_2HPO_4: Disodium hydrogen phosphate

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

B: Boron

O: Oxygen

F: Fluorine

Mg: Magnesium

Al: Aluminium

Si: Silicon

P: Phosphorus

Ca: Calcium

hGFs: Human gingival fibroblasts

ALP: Alkaline phosphatase

S-PRG: Surface pre-reacted glass-ionomer

hDPSCs: Human dental pulp stem cells

DECLARATIONS

- **Ethics approval and consent to participate**

This *in vitro* study was conducted according to the Declaration of Helsinki guidelines and approved by the Ethics Committee of Bezmialem Vakif University (Approval No: E-54022451-050.05.04-95098). Informed consent was obtained from the participants that their extracted teeth would be used in this research.

- **Consent for publication**

Not applicable.

- **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests or financial interests in the companies whose materials are included in this article.

- **Funding**

The authors received no specific funding for this study.

- **Clinical trial number**

Not applicable

- **Authors' contributions**

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Acknowledgement

This study was supported by a grant given by Bezmialem Vakif University Scientific Research Projects Coordination (No: 20230208).

The authors would like to thank Yıldız Technical University Central Laboratory for their support in advanced imaging and analyses.

We are also grateful to Dr. Sevilay Karahan for her valuable support in statistical analysis and to Eng. Gökçenur Hazar for the assistance in the graphical design of the illustrations.

In the preparation of this manuscript, we declare the use of artificial intelligence technology for language editing purposes and including improving the grammar of the text. The AI tools have significantly improved the text's clarity, coherence and overall quality.

We would like to thank Lect. Gamze Gürleyen for her contributions to the language editing and proofreading of this article.

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