The Saudi Dental Journal 36 (2024) 315–320

Contents lists available at ScienceDirect

The Saudi Dental Journal

journal homepage: www.ksu.edu.sa
www.sciencedirect.com

Efficacy of different remineralizing agents on primary teeth exposed to therapeutic gamma radiation: An in vitro randomized control study

Yomna O. Morad [a,*], Shaymaa A. El Shishiny [a], Rania I. Hindi [b], Asmaa A. El Sheshiny [c], Amira M. Samy [d]

[a] Pedodontics and Oral Health department ,Faculty of Dental Medicine for Girls, Al Azhar University, Cairo, Egypt
[b] Conservative Dentistry ,Health Radiation Research Department, The National Center for Radiation Technology Research, Egyptian Atomic Energy Authority, Cairo, Egypt
[c] Physics Department, Faculty of Science for Girls, Al-Azhar University, Cairo, Egypt
[d] Conservative Dentistry Department, Faculty of Oral and Dental Medicine, Badr University in Cairo, Egypt

ARTICLE INFO

Keywords:
Radiotherapy
Remineralization
Mineral contents
SEM–EDX
Diode laser
Bioactive glass

ABSTRACT

Background: Therapeutic radiation-induced caries is a major side effect. Dental and oral heath are adversely affected by the direct effects of radiotherapy. Preventive procedures are preferred to reinforce dental tissue resistance against radiation damage.
Objective: Research aim is to test the impact of different remineralizing protocols on the mineral content of deciduous enamel exposed to a therapeutic dose of gamma radiation.
Methods: Thirty deciduous enamel specimens were divided randomly into three experimental categories based on the type of remineralizing agent used. *Group I* (gamma irradiated teeth, then painted with fluoride varnish), *Group II* (gamma irradiated teeth, then treated with bioactive glass) and *Group III* (gamma irradiated teeth, and then treated with a diode laser 980 nm). Prepared specimens were assessed for mineral content by environmental Energy Dispersive X-ray Analysis. SEM photomicrographs were performed simultaneously. Results were investigated concerning pre- and post-irradiation values difference. The paired-samples *t*-test was performed to compare the atomic and weight percentages of the selected elements obtained from the samples in the control groups before and after therapeutic radiation. One-way analysis of variance and post hoc Tukey tests were used to compare between the categories regarding weight and atomic percentages.
Results: Calcium/Phosphorus (Ca/P) ratio and Fluoride content decreased significantly after radiotherapy. Fluoride content significantly increased after remineralizing agents' application with the greatest increase in Bioactive glass group followed by fluoride varnish (F.varnish) group and the least increase was in diode laser group. Significant increase in Ca/P ratio in bioactive glass group followed by insignificant increase in F.varnish group (I) with the least increase noticed in the Diode laser group.
Conclusion: Therapeutic radiation caused marked decrease in enamel mineral content. However, the reminralizing agents applied have an improving effect on the caused damage.

1. Introduction

World Health Organization has recently noted that oral cancers are to be given priorities. Maxillofacial and neck tumors exist at an increased frequency in humanity, with an incidence rate of 500,000 new patients yearly (Aragón et al., 2022). Although head and neck tumors in children are of a low incidence, the line of treatment, anticipation and prognosis should be considered. Head and neck cancers (HNC) are usually treated by using ionizing radiation, either as alone or combined with chemotherapy (Ligier et al.,2016).

Radiation caries is considered one of the main side effects of radiotherapy in the head and neck region, which weakens teeth, making them brittle and more prone to fractures (de Miranda et al., 2021). Despite diligent oral hygiene practices, and optimal fluoride application, tooth structure in areas exposed to radiotherapy at doses of 60 Gy and more are subjected to an increased risk of dental harmful effects. Fluoride varnish should be applied to high-risk patients for about 2–4 times per year as recommended by the American Dental Association to decrease

* Corresponding author.
E-mail address: yomnaosama.26@azhar.edu.eg (Y.O. Morad).

https://doi.org/10.1016/j.sdentj.2023.11.009
Received 22 August 2023; Received in revised form 3 November 2023; Accepted 5 November 2023
Available online 7 November 2023

Table 1
Remineralizing procedures.

Materials and Devices	Composition	Manufactures
Enamel Pro varnish	5 % Sodium fluoride& 2.3 % Amorphous Calcium Phosphate	Premier Dental Products Co., USA
Bioactive glass Gel (BAG)	45 wt% (wt %) SiO2, 24.5 wt% CaO, 24.5 wt% Na2O and 6.0 wt% P2O5	Experimentally manufactured at Nano Gate company, Cairo, Egypt
Diode laser	Gallium-Aluminium-Arsenide (Ga AlAs) diode laser	SIRONA Dental Company, Germany

Table 2
Comparison of the elemental contents between pre- and post-irradiated groups in terms of weight and atomic percentages.

Groups	Weight (Wt) %		Atomic (At) %	
	Fluoride	Ca/P	Fluoride	Ca/P
Control BR	17.64 ± 1.352	1.990 ± 0.0921	29.15 ± 1.937	1.537 ± 0.05824
Control AR	4.740 ± 1.185	2.568 ± 0.2048	8.84 ± 1.715	2.568 ± 0.2857
Paired *t* test Statistics (*p* value)	<0.0001 *	<0.0001*	<0.0001*	0.0001*

demineralization and increase remineralization (Gupta et al., 2015). Bioactive-Glass has been studied on a wide scale in the field of remineralization due to its enormous capacity to form hydroxyapatite. When it comes in contact with saliva, calcium, sodium, phosphorus, and silicates bind to the enamel surface initiating a remineralizing effect (Klarić et al., 2023). Evidence suggests that laser irradiation can change the makeup of enamel and enhance its resistance to caries because it promotes remineralization and the intake of fluoride ions (Soltanimehr et al., 2019). Therefore, the aim of the current study is to evaluate the effect of different remineralizing agents on the mineral content of enamel of primary teeth exposed to gamma radiation.

2. Materials and methods

This in vitro investigation was conducted by the same operator on 15 exfoliated human primary molars after receiving written consent from the parent that teeth would be used in research purposes. Ethical approval with the number (REC-PD-23-14) was received from the *Research Ethics Committee of the Faculty of Dental Medicine,* girls' branch Al-Azhar University. Collected teeth were visually examined. Teeth with visible carious lesions, hypocalcification, erosion, cracks, previous restorations, teeth exposed to radiation, or subjected to any chemicals such as hydrogen peroxide were excluded. Collected teeth were cleaned using a toothbrush and water to remove any debris and residual tissues, then polished using a non-fluoride polishing paste and brush. Then teeth had been stored in distilled water at room temperature to avoid dehydration. Mesiodistal crown sectioning into equal halves was performed using a low-speed dental diamond disk (DFS, Diamond, Germany). Buccal and lingual enamel samples were then centrally mounted on top of a freshly mixed chemically cured acrylic resin (Acrostone, Egypt) within a prepared mold, with the enamel surfaces facing upward for the experimental design (Table 1).

2.1. Teeth samples grouping

A total of 30 samples, separated into three experimental groups based on the remineralizing agent used, are needed for this investigation, according to the power analysis performed using G*Power 3.1.9.4 software. This sample size guarantees an alpha error (type I error) of 0.05, an effect size of 0.7, and a test power of 0.80.

2.2. Samples exposure to therapeutic gamma radiation (Demineralization Procedure) (Dur et al., 2022)

The specimens underwent irradiation to replicate oral cancer therapy at The National Center for Radiation Research and Technology in Cairo, Egypt, utilizing Indian Cobalt-60. The irradiation was carried out using a Gamma cell chamber (60 Co, Baha, Baha Research Center 4000 A) with a dose rate of 60 Gy (2 Gy × 30 fractional doses), which corresponds to the therapeutic dose for the treatment of (HNC). A specialized technician administered the radiation using a linear accelerator device (Varian Trilogy, Varian Medical Systems, Palo Alto, CA, USA). Irradiated teeth were stored in a saline solution to mimic the oral

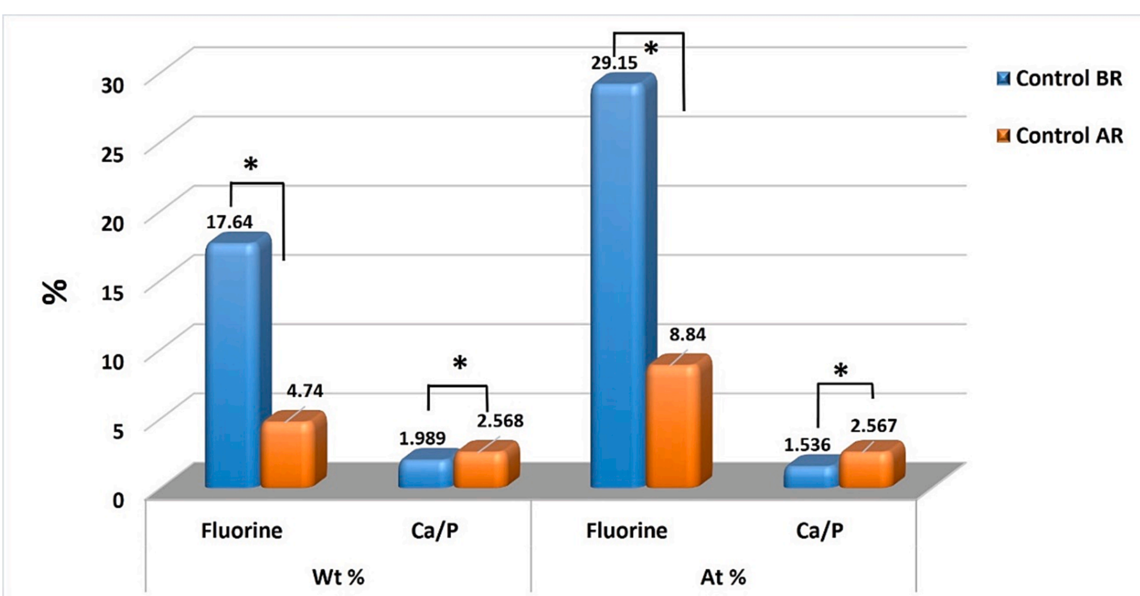

Fig. 1. Weight and atomic percentages of the elemental contents of pre-and post-irradiated groups.

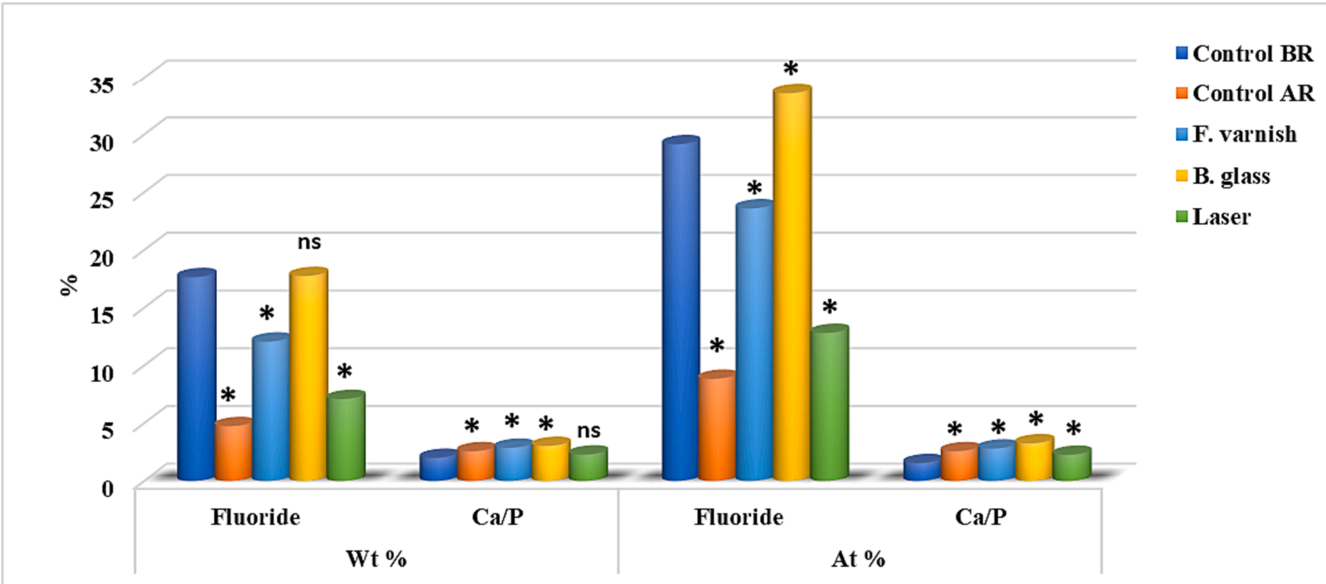

Fig. 2. Weight and atomic percentages of the elemental contents between before, post-irradiated group and other groups that received reminralizing agents.

Table 3
Comparison of the elemental contents before radiation, after radiation and after remineralizing agents' application in terms of weight and atomic percentages.

Groups	Wt %				At %			
	Fluoride	P value vs. control BR	Ca/P	P value vs. control BR	Fluoride	P value vs. control BR	Ca/P	P value vs. control BR
Control BR	17.64 ± 1.352	–	1.989 ± 0.092	–	29.15 ± 1.937	–	1.536 ± 0.058	–
Control AR	4.740 ± 1.185*	<0.0001	2.568 ± 0.2048*	0.0001	8.84 ± 1.715*	<0.0001	2.568 ± 0.2857*	<0.0001
F. varnish	12.06 ± 0.7393*	<0.0001	2.866 ± 0.2703*	<0.0001	23.60 ± 2.296 *	0.0002	2.796 ± 0.1649 *	<0.0001
B. glass	17.74 ± 0.881 ^{ns}	0.9999	3.027 ± 0.1993*	<0.0001	33.57 ± 1.393 *	0.0026	3.252 ± 0.2410 *	<0.0001
Laser	7.087 ± 1.483 *	<0.0001	2.276 ± 0.1184 ^{ns}	0.094	12.81 ± 1.690 *	<0.0001	2.254 ± 2.254 *	<0.0001

(*) in the table indicates a statistically significant difference from the post-irradiated group whereas (ns) indicates a non-significant difference).

environment between applications. Following gamma irradiation, 30 samples were randomly allocated into three experimental groups (n = 10) based on the remineralizing protocol followed in the study design.

Study design:

Control before radiation (BR): Specimens not subjected to therapeutic radiation

Control after radiation (AR): Gamma irradiated samples.

Group I: Gamma irradiated samples, then treated with fluoride varnish.

Group II: Gamma irradiated samples, then treated with experimental bioactive glass.

Group III: Gamma irradiated samples, then treated with diode laser.

2.3. Remineralizing procedures (Table 1)

2.3.1. Fluoride varnish Application

A thin coat was evenly painted on the enamel specimens with a sweeping brush and left undisturbed for 24 h.

2.3.2. Experimental bioactive glass gel Application

A thin gel layer was evenly painted on the teeth samples with a micro brush and left undisturbed for 24 h.

2.3.3. Diode laser Irradiation

Diode laser Gallium-Aluminum-Arsenide (Ga Al As), (Siro-Laser Advance class III b) of 970-nm wavelength and a power output of 2 W was used in continuous mode on the targeted surfaces for 15 s. It was attached to a 220-μm optic conductor fiber as a transmission element. The tip of the optic fiber was placed at a non-contact mode with a standard distance of 2-mm. It was then moved uniformly and longitudinally over the marked spot (Gupta et al., 2015). The specimens were stored, in deionized water at 37 °C for 24 h before conducting the tests.

2.4. Assessment Methods

2.4.1. Chemical analysis evaluation

The specimens underwent analysis for mineral content initially, after gamma irradiation, and following the application of remineralizing protocols. This assessment involved measuring the content of fluoride, calcium, and phosphorus ions in weight percentage (wt%) and atomic percentage (At%) using Environmental Energy Dispersive X-ray Analysis (EDX) conducted on a Quanta 250 model by FEI Company in the Netherlands.

2.4.2. Surface micromorphology inspection

Selected specimens from each group were scrutinized using an Environmental Scanning Electron Microscope (Quanta FEG-250 model,

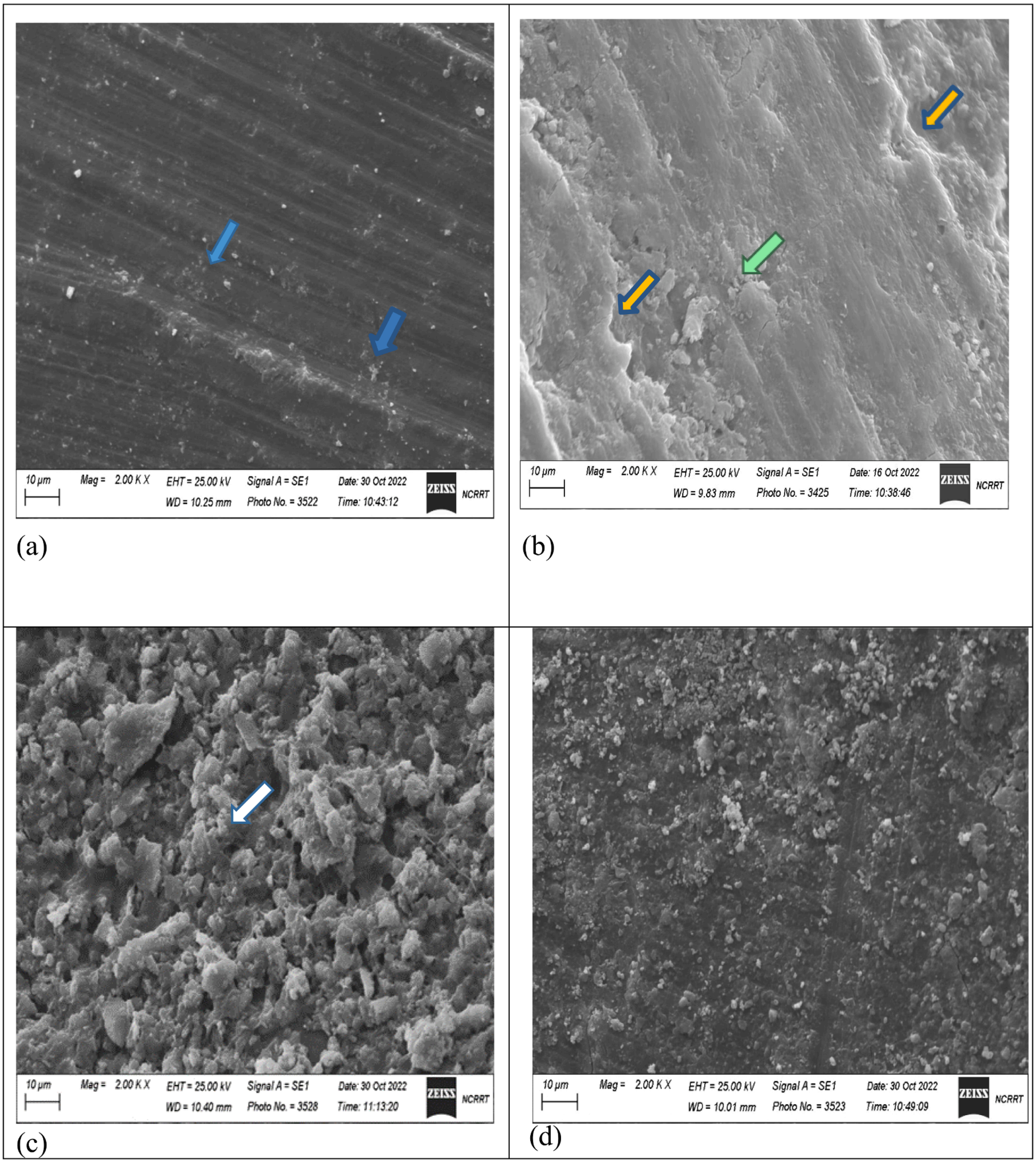

Fig. 3. SEM photomicrograph of enamel surface showing (a) Before radiation [control group], (b) After radiation [control group], (c) Bioactive glass group II, (d) Flouride varnish group I, (e) Diode Laser group III.

Netherlands) at a magnification of 2,000X to observe microstructural changes on the surface.

2.5. *Statistical analysis*

The study data were analyzed on Graph Pad Prism version 8.0.2 software. P. values < 0.05 were regarded as statistically significant. Data were normally distributed in all groups.

The paired-samples *t*-test was used to compare the weight and atomic percentages of some elements obtained from the control group samples before and after irradiation. One-way analysis of variance and post hoc Tukey tests were applied to compare between the study groups in terms of weight and atomic percentages of some elements obtained from the samples in the control groups and groups that received remineralizing agents.

3. Results

Intergroup comparison between post-irradiation and after the application of remineralizing agents has shown a statistically significant difference ($P < 0.001$). Pairwise comparisons between bioactive glass, fluoride varnish, and diode laser groups have shown that fluoride content (wt.%&At%) significantly increased after the application of remineralizing agents, with the greatest increase in the bioactive glass group (II), followed by the fluoride varnish group (I), and the least increase in the diode laser group (III). Regarding the Ca/P ratio, there is a significant increase in the Ca/P ratio after the application of experimental bioactive glass in group (II), followed by an insignificant increase in the

(e)

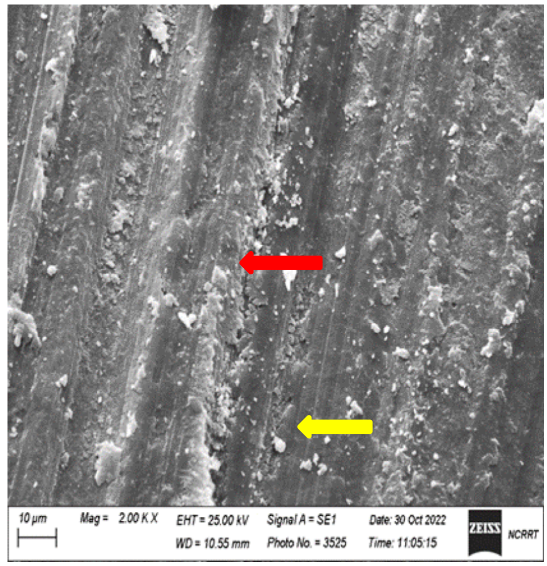

Fig. 3. (*continued*).

fluoride varnish group (I), with the least increase in the diode laser group (III).

Weight and atomic percentage values (mean ± standard deviation) of the elemental contents of the specimens before and after irradiation are shown in Table 2 and Fig. 1. *And between before, post-irradiated groups and after recieving reminralizing agents are shown in* Fig. 2 *and* Table 3 (* in the table and graph indicates a statistically significant difference within before and after radiation whereas ns indicates a non-significant difference).

3.1. SEM Results

Scanning electron photomicrographs of enamel surfaces in different groups are shown in (Fig. 3).

a: Before radiation (control) showed rough enamel surface with parallel ridges (perichymata), some mineral deposits as shown by the blue arrows.

b: After radiation (control) showed rough enamel surface detecting perichymata some areas of enamel showed erosion as shown by the orange arrows.

c: Photomicrograph of bioactive glass group (II) showed more aggregated and more fused mineral precipitation that completely covers the enamel surface. Also precipitation of particles of the material appears in the photomicrograph as denoted by the white arrow.

d: Photomicrograph of flouride varnish group (I) showed areas of mineralization and more mineral aggregation on enamel surface as shown by green arrow compared to control group.

e: Photomicrograph of Diode Laser group (III) showed enamel rods and interrods as well as perichymata, mineral deposition, some minerals appear isolated (yellow arrow), while others appear clustered (red arrow).

4. Discussion

The occurrence of radiation-induced caries poses a significant challenge, potentially leading to rapid deterioration of hard dental tissues. A crucial strategy in preventing tooth decay involves minimizing demineralization and promoting remineralization (Palmier et al., 2020).

The study findings highlighted differences in mineral content values between irradiated enamel and its state before radiation. Radiation induces changes in the mechanical characteristics of hard dental tissues due to factors such as reduced saliva production, which occurs directly or indirectly after radiation (Kouri et al., 2021). It is essential to take precautions to anticipate potential complications during therapeutic doses (Dörr, 2018).

In the present study, no demineralization protocol was done to the specimens to detect the impact of remineralizing protocols against therapeutic radiation's destructive effect. Different devices can be used to determine crystal structure. In the current study, EDX was used for elemental analysis according to (Comar et al., 2018). Bioactive glass, fluoride varnish, and diode laser were used as remineralizing agents. Fluoride and bioactive glass, were delivered as a varnish for sustained release of calcium and fluoride ions for a longer duration (Sleibi et al., 2019). In this study, fluoride varnish layer was undisturbed for 24 h after application, to allow remineralizing ions to be absorbed into the enamel surface (Rodemer et al., 2022).

It was also concluded that bioactive glass was observed to remineralize and heal enamel lesions by filling demineralized areas (Dai et al., 2019), and this agrees with the findings of this study.

Recently, diode lasers have gained prominence in various dental applications. Studies have indicated that low-power lasers offer an alternative approach to cavity prevention (Moharam et al., 2020). Moreover, research has suggested that laser treatment, either alone or in combination with topical fluoride application, can enhance the resistance of teeth to decay. In this study, a 980 nm diode laser was employed due to its capacity to bolster enamel resistance against decay, reduce enamel solubility in acidic environments, and augment enamel surface hardness (Ahrari et al., 2018). Despite primarily being recognized as a soft tissue laser (Romanos, 2015) validated the use of diode lasers on hard dental tissues with the penetration depth of a 980 nm diode laser, that was shallower compared to erbium and Nd:YAG lasers.

In the present study, fluoride content decreased significantly after radiotherapy, while the Ca/P ratio showed a slight increase, aligning with (Velo et al., 2018; Lu et al., 2019). Studies have reported that the application of remineralizing agents after radiotherapy decreases enamel mineral loss and preserves its external crystal structure, but it is somewhat impressive in preserving its mechanical properties (Qing et al., 2015; Hegde et al., 2019).

Regarding the present study, the highest percentage of remineralization was found in Bioactive glass group, which almost returned the mineral content to base line followed by fluoride varnish group, while the lowest percentage was found in diode laser group. The study results were consistent with a previous finding that concluded the remineralizing effect of a toothpaste with bioactive glass on a demineralized enamel surface was significantly higher than the other tested groups (Mohapatra et al., 2019).

Supporting the results of this study, the increased depth of remineralization was found in the Bioactive glass group, which was similar to (Abbassy et al., 2021) who also reported that a sustained fluoride release enhances remineralization. Concerning group III (diode laser), which showed the least increase in mineral content, this was in agreement with those of (Antunes et al., 2004) who stated that reduction in the mineral content after laser application is due to volatilization caused by heat generation. However, they used high-power Nd: a YAG laser, while this study utilized a low-power diode laser (Klarić et al., 2023).

In the present study, SEM observed areas of more aggregated and fused mineral precipitations that completely cover the enamel surface in the bioactive glass group. Additionally, in the fluoride varnish group, there were areas of more mineral aggregation on the enamel surface than in the control sample. In the diode laser group, some mineral depositions appeared isolated while others appeared clustered, compatible with (Thimmaiah et al., 2019).

5. Conclusion

This study demonstrates that radiation leads to alterations in enamel properties. Implementing various remineralization techniques after radiation exposure can mitigate its detrimental impact on dental enamel in patients with head and neck cancer. Bioactive glass has more remineralizing efficacy than fluoride varnish and diode laser.

Recommendation:

More studies are recommended to investigate the effects of experimentally synthesized reminralizing agents.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbassy, M.A., Bakry, A.S., Almoabady, E.H., Almusally, S.M., Hassan, A.H., 2021. Characterization of a novel enamel sealer for bioactive remineralization of white spot lesions. Int. J. Dent. 109.

Ahrari, F., Mohammadipour, H.-S., Hajimomenian, L., Fallah-Rastegar, A., 2018. The effect of diode laser irradiation associated with photoabsorbing agents containing remineralizing materials on microhardness, morphology and chemical structure of early enamel caries. J. Clin. Exp. Dent. 10 (e955).

Antunes, A., Vianna, S., Gomes, A., De Rossi, W., Zezell, D., 2004. Surface morphology, elemental distribution, and spectroscopic changes subsequent the application of nanosecond pulsed Nd: YAG laser on dental enamel surface. Laser Phys. Lett. 2 (141).

Aragón, N., Ordoñez, D., Urrea, M.F., Holguín, J., Collazos, P., García, L.S., et al., 2022. Head and neck cancer in Cali, Colombia: population-based study. Commun. Dent Oral Epidemiol. 50, 292–299.

Comar, L.P., Souza, B.M., Al-Ahj, L.P., Martins, J., Grizzo, L.T., Piasentim, I.S., et al., 2018. Mechanism of action of TiF4 on dental enamel surface: SEM/EDX, KOH-soluble F, and X-ray diffraction analysis. Caries Res. 51, 554–567.

Dai, L.L., Mei, M.L., Chu, C.H., Lo, E.C.M., 2019. Mechanisms of bioactive glass on caries management: a review. Materials. 12 (4183).

de Miranda, R.R., Ribeiro, T.E., da Silva, E.L.C., Júnior, P.C.S., Soares, C.J., Novais, V.R., 2021. Effects of fractionation and ionizing radiation dose on the chemical composition and microhardness of enamel. Arch Oral Bio. 121 (104959).

Dörr, W., 2018. Pathogenesis of normal tissue side effects. In: Basic Clinical Radiobiology. CRC Press, pp. 152–170.

Dur, M.P., Celik, N., Seven, N., 2022. Evaluation of the changes in physical properties and mineral content of enamel exposed to radiation after treating with remineralization agent. Clin. Oral. Investig. 26, 5673–5685.

Gupta, N., Pal, M., Rawat, S., Grewal, M.S., Garg, H., Chauhan, D., et al., 2015. Radiation-induced dental caries, prevention and treatment – a systematic review. Natl. J. Maxillofac. Surg. 6 (160).

Hegde, M.N., Gatti, P., Hegde, N.D., 2019. Protection of wear resistance behaviour of enamel against electron beam irradiation. BDJ Open. 5 (11).

Klarić, E., Tarle, A., Vukelja, J., Soče, M., Grego, T., Janković, B., 2023. Remineralization effects of Er, Cr: YSGG and/or bioactive glass on human enamel after radiotherapy—an in vitro study. Lasers Med. Sci. 38 (65).

Kouri, M., Vadalouca, A., Kouloulias, V., Papadopoulou, E., Vardas, E., Kyrodimos, E., et al., 2021. Oral complications of head and neck cancer therapy. Forum Clin. Oncol.

Ligier, K., Dejardin, O., Launay, L., Benoit, E., Babin, E., Bara, S., et al., 2016. Health professionals and the early detection of head and neck cancers: a population-based study in a high incidence area. BMC Cancer 16, 1–9.

Lu, H., Zhao, Q., Guo, J., Zeng, B., Yu, X., Yu, D., et al., 2019. Direct radiation-induced effects on dental hard tissue. Int. J. Radiat. Oncol. Biol. Phys. 14, 1–11.

Mohapatra, S., Kumar, R.P., Arumugham, I.M., Sakthi, D., Jayashri, P., 2019. Assessment of microhardness of enamel carious like lesions after treatment with nova min, bio min and remin pro containing toothpastes: an in vitro study. Indian J. Public Health. 10.

Moharam, L.-M., Sadony, D.-M., Nagi, S.-M., 2020. Evaluation of diode laser application on chemical analysis and surface microhardness of white spots enamel lesions with two remineralizing agents. J. Clin. Exp. Dent. 12 (e271).

Palmier, N.R., Migliorati, C.A., Prado-Ribeiro, A.C., de Oliveira, M.C.Q., Vechiato Filho, A.J., de Goes, M.F., et al., 2020. Radiation-related caries: current diagnostic, prognostic, and management paradigms. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. 130, 52–62.

Qing, P., Huang, S., Gao, S., Qian, L., Yu, H., 2015. Effect of gamma irradiation on the wear behaviour of human tooth enamel. Sci. Rep. 5, 1–9.

Rodemer, T., Pütz, N., Hannig, M., 2022. Influence of hydroxyapatite nanoparticles on the formation of calcium fluoride surface layer on enamel and dentine in vitro. Sci. Rep. 12 (17612).

Romanos, G., 2015. Current concepts in the use of lasers in periodontal and implant dentistry. J. Indian Soc. Period. 19 (490).

Sleibi, A., Tappuni, A., Karpukhina, N.G., Hill, R.G., Baysan, A., 2019. A comparative evaluation of ion release characteristics of three different dental varnishes containing fluoride either with CPP-ACP or bioactive glass. Dent. Mater. J. 35, 1695–1705.

Soltanimehr, E., Bahrampour, E., Yousefvand, Z., 2019. Efficacy of diode and CO 2 lasers along with calcium and fluoride-containing compounds for the remineralization of primary teeth. BMC Oral. Health 19, 1–9.

Thimmaiah, C., Shetty, P., Shetty, S.B., Natarajan, S., Thomas, N.-A., 2019. Comparative analysis of the remineralization potential of CPP–ACP with Fluoride, Tri-Calcium Phosphate and Nano Hydroxyapatite using SEM/EDX–An in vitro study. J. Clin. Exp. Dent. 11, e1120.

Velo, M.M.A.C., Farha, A.L.H., da Silva Santos, P.S., Shiota, A., Sansavino, S.Z., Souza, A. T., et al., 2018. Radiotherapy alters the composition, structural and mechanical properties of root dentin in vitro. Clinl. Oral. Invest. 22, 2871–2878.