**ORIGINAL ARTICLE**

# The Impact of Radiotherapy in the in Vitro Remineralization of Demineralized Enamel

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## Abstract

**Objective:** To evaluate the impact of radiotherapy on enamel around restorations of glass ionomer cement (GIC) and fluoride tooth paste (FTP). **Material and Methods:** Eighty enamel blocks were made and randomly distributed into two groups, according to the fluoride therapy, non-fluoride tooth paste (NFTP) and FTP (n=40) and in subgroups in conformity with radiation dose (0, 10, 30 and 60 Gy). Roughness and microhardness enamel analyses were conducted before radiotherapy. Enamel cavities were made and restored with two GIC (Ketac Molar Easy Mix or Vitremer). Enamel blocks were submitted to 10, 30 and 60 Gy. Then, artificial enamel caries lesions were created by a pH-cycling procedure and FTP or NFTP were used as treatment. The restored enamel blocks were submitted to final roughness and microhardness analyses. Roughness increase ($\Delta R$) and hardness loss ($\Delta H$) values of enamel were submitted to ANOVA and Tukey test (p=0.05). **Results:** The irradiated enamel group showed statistically higher $\Delta R$ (0.44 ±0.2) and $\Delta H$ (99.26±7.0) values compared to non-irradiated group ($\Delta R$ = 0.051±0.02; $\Delta H$=66.16±12.7) when a resin-modified GIC and NFTP were used. **Conclusion:** Higher radiation dose increased dissolution of bovine enamel. The use of GIC associated with FTP decreased roughness and increased enamel hardness after radiotherapy.

**Keywords:** Dental Enamel; Glass Ionomer Cements; Hardness Tests; Radiotherapy.

**Introduction**

In the recent years, the number of oral and oropharyngeal cancer cases has increased [1]. Patients that have been diagnosed with oral cancer can be treated by several treatment methods such as radiotherapy [2,3], surgery [4], chemotherapy [2,3] or by the combination thereof. Head and neck radiotherapy (HNRT) uses fractioned radiation doses applied daily to the patient to control the tumor mass [5]. In the oral cavity, the most frequent and undesirable side-effects of the above-mentioned cancer therapy are xerostomia, mucositis, candidiasis, dysgeusia, muscular trismus, vascular alterations, osteoradionecrosis and radiation related caries (RRC) [6].

RRC is characterized by a fast onset, progressing quickly and occurring in unusual dental areas such as the cervical and incisal edges of the tooth enamel [7,8]. Active carious lesions are generally seen at the end of the radiation treatment, or few months later, depending on the oral hygiene habits of the patient. When no preventive treatment is performed, an excessive damage of the dentition is commonly seen within the first year after the patient undergoes the radiation therapy [9]. This form of dental caries is a complex and multifactorial disease [10,11] and is related to many factors such as the radiogenic damage of the salivary glands, leading to post-radiation hyposalivation, the increase of cariogenic bacteria, poor oral hygiene and the intake of a more cariogenic diet after HNRT [10,12]. In addition, the direct radiogenic destruction of the mineralized dental structure should be considered a RRC pathogenesis [13]. Scientific evidences have reported alterations in the enamel microstructure, showing an increased dissolution of the dental hard tissue after radiotherapy [14-17]. However, there is limited and contradictory information regarding the direct effects of HNRT on the dental structure, causing RRC in the tooth enamel and dentin [10,15-21].

Unfortunately, the clinical management of RRC is based on the clinical experience of the clinicians [10,22,23]. There is no specific restorative dental treatment and preventive protocol for patients who undergo HNRT based on scientific evidence. It is widely known that fluoride use plays an important role in the prevention of dental caries, as well as on the improvement of the micromechanical properties of the dental hard tissues [24,25]. Therefore, fluoride-releasing materials and topical fluoride are indicated to reduce the caries risk in dental restorations of irradiated patients [26-28]. Nevertheless, it is not totally clear if both strategies cause significant therapeutic effect in the enamel after the exposure to several radiotherapy doses. Therefore, this research study aimed evaluating the impact of radiotherapy (RDT) in the enamel around glass ionomer cement restoration (GIC) and in fluoride toothpaste (FTP) by evaluating the microhardness and the surface roughness of bovine tooth enamel.

The null hypotheses established for this study were the following: 1) There is no impact of radiotherapy in the microhardness and the surface roughness of bovine tooth enamel 2) The use of glass ionomer cement restorations and fluoride toothpaste does not have protective effect to caries in irradiated tooth enamel.

## Material and Methods

Sample Preparation

Eighty freshly extracted bovine incisor teeth stored in 0.5% thymol solution at 4°C were used in this research study. The teeth were horizontally cut in the cervical and incisal areas, using a water-cooling diamond blade (South Bay Technology Inc., San Clemente, CA, USA). Then, one central fragment was longitudinally cut to obtain an enamel block with the following measurements: 4 mm width, 4 mm height and 4 mm thickness. After sectioning the tooth, the dental block dentin was wet-grounded with a silicon carbide paper (#320) to a 2 mm thickness. The enamel surface was wet-grounded with a series of silicon carbide papers (#600 and #1.200) to achieve a flat surface. Subsequently, the enamel blocks were embedded in autopolymerizing acrylic resin (Vipi Produtos Odontológicos, São Paulo, SP, Brazil) and stored at 4°C.

In order to simulate the in vitro remineralization of the demineralized-irradiated enamel around glass ionomer cement restorations, circular cavities were made on the enamel surface and restored with two glass ionomer cements - Ketac Molar Easy Mix  or Vitremer (3M ESPE Dental Products, St Paul, MN, USA). Then, the enamel blocks were submitted to simulated radiotherapy, applying several ionizing radiation doses as 10, 30 and 60 Grays (Gy). Demineralizing and remineralizing solutions (pH cycling) and fluoride therapy [fluoride toothpaste (FTP) or non-fluoride toothpaste (NFTP)] were applied on the enamel surface. Surface roughness and microhardness analyses of bovine tooth enamel were performed to calculate the roughness increase ($\Delta R$) and hardness loss ($\Delta H$) values. The composition and the batch number of each product are shown in Table 1.

**Table 1. Composition and batch numbers of materials used.**

| Product (Batch number) | Manufacturer | Composition |
|---|---|---|
| Ketac™ Molar Easymix Lot. 374638 | 3M ESPE Dental Products | Water, copolymer of acrylic acid-maleic acid, tartaric acid |
| Vitremer™ Lot. 0821000456 | 3M ESPE Dental Products | Silane treated glass, potassium persulfate, water, copolymer of acrylic and itaconic acids, 2-hydroxyethyl methacrylate (HEMA), ethyl acetate |
| Colgate Máxima Proteção Anticáries® | Colgate | 1500ppm of fluoride, Calcium Carbonate, Sodium Lauryl Sulfate, sodium saccharin, tetrasodium pyrophosphate, sodium silicate, polyethylene glycol, sorbitol, carboxymethyl cellulose, methyl paraben, propyl paraben, flavor composition, water and sodium Monoflourfosfato - MPF® |
| Creme Dental Phillips® | Glaxo Smithkline | Calcium carbonate, Sorbitol, Magnesium Hydroxide, Sodium lauryl sulfate, carboxymethylcellulose, mint flavor, magnesium sulfate, saccharin, and purified water. |
| Demineralizing Solution | Roval (Manipulation Pharmacy) | 2 mM calcium chloride, 2 mM potassium phosphate, 75 mM sodium acetate - pH 4.3 |
| Remineralizing Solution | Roval (Manipulation Pharmacy) | 1.5 mM calcium chloride; 0.95mM potassium phosphate; 150mM potassium chloride - pH 7.0 |

Eighty enamel blocks were prepared for restoration, applying restorative materials (Ketac Molar Easymix or Vitemer), being randomly distributed into two groups, according to the fluoride

therapy (FTP and NFTP) (n=40) and, into four subgroups, according to the ionizing radiation dose (0 Gy, 10 Gy, 30 Gy and 60 Gy) (n=10).

Initial Surface Roughness and Microhardness Analyses

After 24 hours following the storage period, the initial surface roughness analysis of the enamel blocks was performed in a rugosimeter (Surftest SJ-301; Mitutoyo Corp., Kanagawa, Japan). Three measurements were randomly taken on the enamel surface, following the test conditions: Lc – 0.25 mm and 0.5 mm/s speed. The measurements were the arithmetic mean between peaks and valleys (Ra), obtained by the path described by the mechanical probe. Three measurements were taken in each enamel block and the arithmetic mean was calculated, obtaining the initial roughness values (ΔRinitial).

The initial Vickers hardness (ΔHinitial) was measured using a Vickers impression tester (15s indentation time, 100g load, HMV-2, Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan). Five indentations were randomly made on the enamel surface and the length of the diagonals (d1 and d2) left by the indenter was digitally measured using microscope light (HMV-2 microhardness tester at 50x magnification). Subsequently, the initial microhardness values (Δinitial) were calculated.

Cavity Preparation and Restoration Placement

Circular cavities (1.5 mm in diameter and 1.5 mm in depth) were made in the center of the enamel surface using diamond burs (n°. 2294, KG Sorensen, Cotia, SP, Brazil) placed in high-speed handpieces (Dabi Atlante, Ribeirão Preto, SP, Brazil) under water-cooling.

The cavities were filled with restorative materials, according to two groups: conventional glass ionomer cement (Ketac Molar Easy Mix) and resin-modified glass ionomer cement (Vitremer). Both glass ionomer cements were handled following the manufacturer's instructions. The material that underwent physical polymerization (resin-modified glass ionomer cement) was exposed to visible light, for 40 seconds, with 600 mW/cm2 intensity (Optilux Plus, Gnatus Equipamentos Médico-Odontológicos, Ribeirão Preto, SP, Brazil). Glass ionomer cement restorations received surface protection and were kept in a humidifier for 30 minutes. After this period, the restorations were polished using flexible disks (SofLex Pop-On, 3M ESPE, St. Paul, MN, USA), following a descending order of granulometry and the enamel blocks were stored in deionized water for 24 hours at 37°C.

Simulated Radiotherapy

The specimens were submitted to simulated radiotherapy applied in a single session according to the ionizing radiation dose (10 Gy, 30 Gy and 60 Gy). Primus K Linear Accelerator (Siemens Medical Solutions USA, Inc., Malvern, PA, USA) with 6 MeV power, 100 cm source surface distance and field size 18 cm × 23 cm was used in the study.

Artificial Caries Induction by pH-Cycling

Artificial enamel caries was created by a pH-cycling procedure, modified from the previously described protocol [29]. Each specimen was cycled in 15 ml demineralizing solution for 6 hours. Subsequently, the enamel blocks were washed with distilled water and submitted to treatment (FTP or NFTP) for 5 minutes. Then, the samples were washed with distilled water and immersed in 15 ml remineralizing solution for 18 hours. This procedure was carried out for 14 days at room temperature under no stirring process. Ten cycles were performed for each experimental group. During the cycles, the solutions were daily removed, except in the 6th, 7th, 13th and 14th days, when the samples were kept in remineralizing solution. After undergoing the pH-cycling, the enamel blocks were kept in distilled water at 37°C for final roughness and microhardness analyses.

Final Roughness and Microhardness Analyses

The final roughness ($\Delta Rfinal$) and microhardness values ($\Delta Hfinal$) were obtained after the 24-hour storage period, following the same test conditions as the initial roughness and microhardness analyses.

Statistical Analysis

The difference between the final and the initial roughness ($\Delta Rfinal - \Delta Rinitial$) was calculated to obtain the roughness increase ($\Delta R$). Likewise, the difference between the final and the initial microhardness ($\Delta Hfinal - \Delta Hinitial$) was calculated to obtain the values for hardness loss ($\Delta H$). Normality and homoscedasticity data was assessed applying the Shapiro Wilks test at a preset alpha of 0.05. The roughness increase and the hardness loss values were subjected to the three-way ANOVA, Tukey post-hoc test at a significance level of 5% - IBM SPSS Statistics for Windows Software, version 20 (IBM Corp., Armonk, NY, USA).

**Results**

Statistical reports have shown that the factors 'material', 'radiation dose', 'fluoride therapy' and all the possible interactions between them have presented statistically significant values for roughness increase and hardness loss (p<0.0001). The $\Delta R$ and $\Delta H$ values are presented in Table 2 and Table 3.

The $\Delta R$ values of bovine enamel around glass ionomer cement restorations were statistically higher for the irradiated groups (10, 30 and 60 Gy) compared to the control group (0 Gy), when NFTP was used. In contrast, there were no statistical differences between the $\Delta R$ values, for the control and the irradiated groups, after the application of FTP. Bovine enamel presented statistically lower $\Delta R$ values after the use of FTP compared to NFTP for every ionizing radiation doses in both dental cements. When conventional glass ionomer cement (Ketac Molar Easy Mix) was used as a restorative material, bovine enamel showed statistically lower $\Delta R$ values compared to the enamel on resin-modified glass ionomer cement (Vitremer) for 10 and 30 Gy, when NFTP was used (Table 2).

**Table 2. Means values of the enamel roughness increase (ΔR), comparing glass ionomer cements, with different ionizing radiation doses and fluoride therapy.**

| Material | Dose of Ionizing Radiation (Gy) | Fluoride Therapy | |
|---|---|---|---|
| | | Non-Fluoride Toothpaste (NFTP) | Fluoride Toothpaste (FTP) |
| Ketac Molar | Control (0 Gy) | 0.068±0.02Aa | 0.042±0.01Aa |
| | 10 Gy | *0.242±0.07Bb | 0.04±0.007Aa |
| | 30 Gy | *0.35±0.09Bbc | 0.05±0.02Aa |
| | 60 Gy | 0.42±0.1Bc | 0.058±0.01Aa |
| Vitremer | Control (0 Gy) | 0.051±0.02Aa | 0.038 ±0.01Aa |
| | 10 Gy | 0.39±0.1Bb | 0.042 ±0.01Aa |
| | 30 Gy | 0.52± 0.09Bb | 0.045 ±0.01Aa |
| | 60 Gy | 0.44 ±0.2Bb | 0.11 ±0.09Ab |

Means followed by different letters (uppercase on the same row and lowercase on the same column) indicate statistical difference for each glass ionomer cement; *Ketac Molar differs from Vitremer in the same radiation dose and fluoride therapy conditions; α=0.05.

The enamel ΔH values presented no statistical difference comparing the irradiated groups (10, 30 and 60 Gy) to the control group (10 Gy), when the conventional glass ionomer cement (Ketac Molar Easy Mix) was used in both fluoride therapies. For resin-modified glass ionomer cement (Vitremer), the enamel ΔH values showed no statistical difference comparing the control group to all the radiation doses, only for FTP.

The bovine enamel around Ketac Molar Easy Mix showed statistically higher ΔH values for NFTP compared to FTP after the application of 60 Gy. The enamel around Vitremer presented higher ΔH values for NFTP compared to FTP in all doses of ionizing radiation (Table 3). The enamel around conventional glass cement ionomer (Ketac Molar Easy Mix) showed statistically lower ΔH values than resin-modified glass ionomer cement (Vitremer) for all the ionizing radiation doses (0 Gy, 10 Gy, 30 Gy and 60 Gy), when NFTP was used (Table 3).

**Table 3. Means values of the enamel hardness loss (ΔH), comparing glass ionomer cements, with different ionizing radiation doses and fluoride therapy.**

| Material | Dose of Ionizing Radiation (Gy) | Fluoride Therapy | |
|---|---|---|---|
| | | Non-Fluoride Toothpaste (NFTP) | Fluoride Toothpaste (FTP) |
| Ketac Molar | Control (0 Gy) | *43.51±10Aa | 32.73±10.4Aa |
| | 10 Gy | *45.37±7.9Aa | 32.23±9.4Aa |
| | 30 Gy | *46.23±1.5Aa | 33.99±7.0Aa |
| | 60 Gy | *53.50±10.8Aa | 33.20±6.1Ab |
| Vitremer | Control (0 Gy) | 66.16±127.Ab | 410.7±9.9Aa |
| | 10 Gy | 70.30±13.6Ab | 44.44±9.9Aa |
| | 30 Gy | 70.80±9.5Ab | 452.9±78.Aa |
| | 60 Gy | 99.26±72.Bb | *572.9±15Aa |

Means followed by different letters (lowercase on the same row and uppercase on the same column) indicate statistical difference for each glass ionomer cement; *Ketac Molar differs from Vitremer in the same radiation dose and fluoride therapy conditions; α=0.05.

## Discussion

This study aimed at verifying the impact of radiotherapy in the in vitro remineralization of demineralized bovine tooth enamel by evaluating the increase of the roughness and the microhardness loss of enamel around glass ionomer restorations after the application of several doses of ionizing radiation (10, 30 and 60 Gy) and fluoride therapy. Therefore, based on our results,

simulated radiotherapy may change the enamel properties, resulting in higher risk of dental demineralization and degradation. This fact is mainly seen when a higher radiation dose (60 Gy) is applied. Furthermore, our results have shown that conventional glass ionomer cement associated to the daily use of FTP may decrease the roughness and increase the microhardness values. Thus, both null hypotheses of the current study were rejected.

Head and neck radiotherapy consists of a total high-energy x-ray radiation dose varying from 50 to 80 Gy, applied in daily fractions of 1.8 to 2 Gy [13,22]. There is a direct correlation between the tooth destruction severity and the radiation dose that reaches the dental structure [13]. Previous studies have reported that the direct radiotherapy effects produce degradation of the enamel organic matrix via proteolysis of the non-collagenous proteins that are highly radiosensitive [14,30,31]. Ionizing radiation also acts on water, leading to the formation and accumulation of free radicals and reactive oxygen species, which may oxidize and denature the organic components of the dental structure [15,32]. Indirect radiogenic effects on inorganic components have also been identified, such as microcracks in the hydroxyapatite crystals [33,34]. Our results have shown that irradiated-demineralized enamel presents higher $\Delta R$ values compared to non-irradiated bovine enamel (control) when NFTP was used (Tables 2 and 3). These results may suggest that ionizing radiation doses cause alterations in the inorganic and/or organic components, increasing the dissolution of the enamel surface, agreeing with the results reported by additional studies [15-17]. This finding may suggest the implementation of clinical preventive strategies since radiotherapy-induced enamel defects may establish easier colonization of cariogenic bacteria, increasing the RRC and the secondary caries risk in cancer patients who undergo radiotherapy.

It is important to mention that single radiation doses were used in the present study and the destructive effect on the enamel microstructure may be overestimated. Nevertheless, the enamel samples were also submitted to lower radiation doses (10 and 30 Gy) in order to simulate the radiotherapy effect on teeth located contra-laterally to the irradiated tumor that received lower radiation dose [35]. However, alterations to the enamel microhardness were observed only after the application of a higher ionizing radiation dose (60 Gy). This fact may occur because direct radiogenic effects happen when higher doses are applied and, also, due to the roughness higher sensibility when compared to the microhardness analysis to measure the initial enamel alterations, after the radiation and the demineralization process.

During the HNRT, a continuous follow-up of the patient and the use of fluoride-releasing materials are necessary to reduce the side-effects caused by treatment to the dental hard tissues, aiming at improving the patients' quality of life [36]. This study used two-glass ionomer cements (Ketac Molar Easy Mix and Vitremer) as restorative materials in combination with FTP and NFTP. In this research study, the in vitro results have shown no statistical difference for the $\Delta R$ and $\Delta H$ values comparing the non-irradiated and irradiated enamel samples after the application of FTP for both glass ionomer cements. This finding may be explained as the fluoride ions released from the restorative materials may substitute the hydroxyl ions from the enamel hydroxyapatite, leading to

the formation of fluorapatite crystals, which are less susceptible to dissolution [37,38]. Thus, the combination of FTP and glass ionomer cements presented a protective effect, decreasing the direct alterations on the enamel properties after radiotherapy.

In this study, the ΔR and ΔH values of irradiated enamel were similar to the values of non-irradiated enamel when conventional glass ionomer cement was used as restorative material, regardless of the toothpaste. On the other hand, the resin-modified glass ionomer cement (Vitremer) presented beneficial effect on demineralized-irradiated enamel only when FTP was used. Furthermore, our results showed that the bovine enamel adjacent to the conventional glass-ionomer cement restorations (Ketac Molar Easy Mix) presented statistically lower ΔR and ΔH values compared to bovine enamel around resin-modified glass ionomer cement (Vitremer) when associated to NFTP, as observed in most groups. This fact may be explained because conventional glass ionomer cement presents higher solubility, releasing a greater amount of fluoride ions from its structure and, consequently, decreasing the enamel demineralization [39] as reported in additional studies [39,40].

The implementation of preventive oral health care programs in head and neck cancer patients, who underwent radiotherapy, is extremely important to achieve better results in dental restorative procedures, as well as to improve their quality of life [22]. Our findings have reinforced the importance of using topical fluoride therapy during and after HNRT, suggesting that its synergistic use with conventional GIC promotes more significant effects on the remineralization of irradiated enamel. However, it is important to emphasize that the positive effect of topical fluoride therapy on the dental structure was verified under in vitro conditions in this study. Therefore, our results should be confirmed by randomized controlled trial studies to shown that this therapy produces the remineralization of irradiated enamel. Furthermore, GIC are water-based dental cements and their physical properties are negatively affected when used as restorative materials in a dry oral environment, typically found in head and neck cancer patients after undergoing radiotherapy sessions [41].

A limitation of this study is that the oral conditions of irradiated patients, including salivary pH and dietary changes, were not simulated. Hence, more research efforts are necessary to develop bio-active restorative materials featuring desirable characteristics such as higher solubility resistance in post-radiation oral conditions, chemical bond strength to the dental structure and optimal mechanical properties.

## Conclusion

A higher dose of ionizing radiation (60 Gy) has impaired the remineralization process of demineralized bovine enamel, increasing the surface roughness and decreasing the microhardness when a non-fluoride toothpaste was used. The use of GIC associated to FTP has decreased the roughness and increased the enamel hardness after being submitted to simulated radiotherapy sessions.

**Conflict of Interest:** The authors declare no conflicts of interest.

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