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Effect of gamma irradiation and restorative material on the biomechanical behaviour of root filled premolars

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Abstract

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Aim To investigate *ex vivo* the influence of gamma irradiation therapy and restorative material on fracture resistance, fracture mode and strain of root filled human premolars.

Methodology Sixty extracted human maxillary premolar teeth were randomly divided into six groups ($n = 10$) determined by two study factors: (i) restorative materials: sound teeth, root filled teeth restored with composite resin, root filled teeth restored with amalgam; (ii) gamma irradiation: irradiated (subjected to 60 Gy of gamma irradiation in daily increments of 2 Gy) and nonirradiated. For the strain gauge test, two strain gauges per sample were attached on the buccal and palatal cusp surfaces ($n = 5$). Strain values were recorded during loading of 0–150 *N*. Fracture resistance (*N*) was assessed in a mechanical testing machine ($n = 10$). Strain gauge for each cusp and fracture resistance data were analysed by two-way ANOVA (3×2) followed by the Tukey's honestly significant difference test ($\alpha = 0.05$). The failure mode was evaluated using an optical stereomicroscope and classified according to the location of the failure.

Results Gamma radiation therapy significantly reduced the fracture resistance of intact teeth. The strain was higher for teeth restored with amalgam than for those restored with composite resin. The teeth restored with composite resin had similar strain values to sound teeth. Nonirradiated teeth had more restorable failures than irradiated teeth.

Conclusions Gamma irradiation significantly reduced fracture resistance and increased cusp strain. The use of composite resin resulted in better biomechanical behaviour than amalgam for restoring root filled teeth whether or not they were submitted to radiotherapy.

Keywords: failure mode, fracture strength, radiotherapy, root filled teeth, strain gauge method.

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Introduction

Patients, when diagnosed with head and neck cancer, may be treated by surgical management (Hong *et al.* 2001), radiotherapy (Lazarus *et al.* 2007), chemotherapy (Lyons 2006) or by a combination of these therapies. Despite the advantage of preserving the structure of tissues, radiotherapy causes adverse reactions in the oral cavity (Kielbassa *et al.* 2006).

It is known radiotherapy contributes to a high incidence of tooth destruction, mainly because of radiation caries (Kielbassa *et al.* 2006). Studies have reported the occurrence of physical and chemical changes (Aoba *et al.* 1981, Baker 1982) that modify

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the mechanical properties (Kielbassa *et al.* 1997, al-Nawas *et al.* 2000, Soares *et al.* 2010) and decrease the fracture resistance of irradiated teeth (Aoba *et al.* 1981, Baker 1982). Head–neck radiotherapy also increases root canal microflora diversity with an increasing risk of apical periodontitis (Hommez *et al.* 2008). Moreover, irradiated pulps undergo nuclear alterations, such as fibrosis and hyaline degeneration (Vier-Pelisser *et al.* 2007). Root canal treatment and direct restorations are frequently indicated for restoring teeth with compromised coronal structure before head–neck radiotherapy.

Root canal treatment might compromise the biomechanical properties of teeth (Trope *et al.* 1986, Cobankara *et al.* 2008). Root filled teeth are more susceptible to fracture as a result of increased brittleness (Sathorn *et al.* 2005) and are often weakened as a result of coronal destruction from dental caries (Hürmüzlü *et al.* 2003), access cavity preparation, instrumentation of the root canal, loss of moisture in dentine and previous restorations or endodontic therapy (Tang *et al.* 2010).

In respect of therapeutic approaches, amalgam has been characterized as technically easy to use and clinically predictable (Mondelli *et al.* 1998). However, amalgam does not bond to tooth structure (Assif *et al.* 1990). When restoring with amalgam, the original stress distribution of the restoration is not recovered (Versluis & Tantbirojn 2006). Moreover, if the amalgam does not adhere to the tooth structure, then an incomplete recovering of fracture resistance to the tooth structure is associated with higher cusp deformation under compressive loads (Reeh *et al.* 1989, Assif *et al.* 1990). Several studies have shown that the restoration of root filled posterior teeth results in higher fracture resistance when adhesive restorative materials, such as composite resins, are used, as they reinforce the remaining tooth tissues (Lohbauer *et al.* 2003). Changes created in the organic and mineral tissues by radiation therapy (Soares *et al.* 2010) should be considered when restoring irradiated teeth. However, no study has ever simultaneously investigated the effect of the gamma irradiation and the restorative material on the biomechanical behaviour of root filled teeth.

Therefore, the aim of this laboratory study was to evaluate the effect of the Co-60 gamma radiation and restorative material (amalgam or resin) on fracture resistance, failure mode and strain of root filled human premolars. The null hypothesis is that these factors have no effect on the biomechanical behaviour of root filled human premolars.

Materials and methods

Specimen preparation

Sixty sound human single-rooted maxillary premolars, extracted for periodontal reasons, free from cracks and defects were selected (approved by the Federal University of Uberlândia Ethics Committee, No. 211/2005). Teeth of similar size and shape were selected by crown dimensions after measuring the buccolingual and mesiodistal widths in millimetres, allowing a maximum deviation of 10% from the determined mean (Soares *et al.* 2008c). Roots were then embedded in self-polymerizing polystyrene resin (AM 190; AeroJet, Santo Amaro, SP, Brazil) to a level 2.0 mm below the coronal limit (Soares *et al.* 2005). The periodontal ligament was simulated using a polyether-based impression material (Impregum Soft; 3M-ESPE, St. Paul, MN, USA). To carry out this procedure, root surfaces were dipped into molten wax (Epoxiglass, Diadema, SP, Brazil) up to 2.0 mm apically to the coronal surface, resulting in a 0.2- to 0.3-mm-thick wax layer. A radiographic film with a centralized circular hole was used to stabilize teeth for the embedding procedure. This assembly was placed with the crown faced down into a hole in a wooden board leaving the root in a vertical position perpendicular to the supporting radiographic film. Then, a plastic cylinder (Tigre, Rio Claro, SP, Brazil), 20 mm in height and 22 mm in diameter, was placed over the root and fixed in position with cyanoacrylate-based adhesive (Super Bonder; Loctite, Itapevi, SP, Brazil) and wax. The polystyrene resin was manipulated according to the manufacturer's instructions and inserted into the cylinder. After resin polymerization, the teeth were removed from the cylinder and the wax was removed from both the root surface and the resin cylinder. The polyether material was placed in the resin cylinder, the tooth was inserted in the cylinder, and the excess polyether material was removed with a scalpel blade (Soares *et al.* 2005). Teeth were randomly distributed into six groups ($n = 10$) defined by the two factors in the study: tooth condition factor (S, sound teeth; RF-RC, root filled teeth restored with composite resin; RF-AM, root filled teeth restored with amalgam) and radiotherapy factor (Ir, irradiated; NIr, nonirradiated). Teeth were stored in distilled water and 0.2% thymol solution at 37 °C and prepared as follows:

Sound, nonirradiated teeth

Sound nonirradiated teeth without root treatment were used as controls.

Root filled, nonirradiated teeth restored with composite resin

Root canal treatment was performed, followed by a mesio-occlusal-distal (MOD) preparation and a composite resin restoration. Root canals were manually enlarged with size 10–50 master apical files (K-files; Dentsply Maillefer, Ballaigues, Switzerland). A step-back technique was used with stainless steel K-files, Gates-Glidden drills 2–4 (Dentspl, Maillefer) and 2.5% sodium hypochlorite irrigation (Miyako, Guarulhos, SP, Brazil). The roots were filled with gutta-percha points and calcium hydroxide-based cement (Sealer 26; Dentsply, Petrópolis, RJ, Brazil). Then, a MOD cavity was prepared using a standardized preparation machine (Federal University of Uberlândia, Uberlândia, MG, Brazil) (Soares *et al.* 2008a). The device consists of a high-speed handpiece (KaVo do Brasil Ltd, Joinville, SC, Brazil) coupled to a mobile base. The mobile base moves vertically and horizontally, in increments of 3 μm, with the aid of a micrometer (Mitutoyo, Tokyo, Japan) accurate to a level of 0.1 mm (Soares *et al.* 2006, 2008a). For the MOD cavity preparation, an inverted cone diamond rotary cutting instrument (No. 1151; KG Sorensen, Barueri, SP, Brazil) was used to prepare the teeth to 1/3 of the intercuspal distance measured at the isthmus cavosurface angle, 2.5 mm deep in the occlusal region and 4 mm deep in the proximal boxes. Teeth were etched with 37% phosphoric acid gel (Cond AC; FGM, Joinville, SC, Brazil) for 15 s, rinsed with water spray and gently dried with absorbent paper. Two applications of a one-bottle adhesive system (Adper Single Bond 2; 3M-ESPE) were applied with an interval of 20 s and light polymerized for 20 s with a halogen light unit with an intensity of 800 mW cm^{-2} (XL 3000; 3M-ESPE). The composite resin (Filtek Z250; 3M-ESPE) restorations were performed with increments of 2.0 mm thickness light polymerized for 40 s. After 24 h of storage in distilled water at 37 °C, finishing of restorations was performed with fine and extra-fine diamond burs (KG Sorensen) in a low-speed handpiece with water spray. Polishing was then performed with silicon carbide discs (Soft-Lex; 3M-ESPE).

Root filled, nonirradiated teeth restored with amalgam

Root canal treatment was performed, followed by a MOD cavity preparation and an adhesive procedure combined with an amalgam restoration (Sagsen & Aslan 2006). The dispersion phase high-copper amalgam capsules (Permite C; SDI, Bayswater, Australia) were mechanically mixed in an amalgamator (Astron Mix; Dabi Atlante, SP, Brazil) for 8 s, and the amalgam was inserted, condensed, burnished and carved into the cavity.

Sound teeth and irradiated

Sound teeth were submitted to the radiotherapy protocol with 60 Gy of Co-60 gamma radiation fractionated into 2 Gy daily, 5 days per week. The dose was defined on the radiotherapy unit panel that self-measures the radiation level emitted. The samples were stored in distilled water changed daily, before and during the irradiation procedure. This protocol is the same as that used in patients under oncogenic treatment for head and neck tumours and was applied in a specialized cancer centre (Uberlândia Cancer Hospital, Federal University of Uberlândia, MG, Brazil) with a Co-60 teletherapy unit (Theratron Phoenix External Beam Therapy System; Best Theratronics Ltd., Ottawa, ON, Canada).

Root filled, irradiated teeth restored with composite resin

Root canal treatment was performed as described before, and teeth were submitted to the irradiation protocol performed in the sound teeth and irradiated group, then the MOD cavity preparation and composite resin restoration were completed.

Root filled, irradiated teeth restored with amalgam

Root canal treatment was performed, and teeth were submitted to the irradiation protocol performed in the sound teeth and irradiated group, then the MOD cavity preparation and amalgam restoration were completed.

To measure tooth strain on the gingivo-incisal direction, two strain gauges (PA-06-060BG-350L; Excel Sensores, Embú, São Paulo, Brazil) were attached to five specimens of each group parallel to the long axis of each specimen, one on the buccal cusp surface and the other on the palatal. The strain gauge grid had an area of 4.1 mm^2, with an electrical resistance of 350 Ω and a gauge factor of 2.12, which is a proportional constant between electrical resistance variation and strain. For the strain gauge attachment, the root surface was etched with 37% phosphoric acid for

15 s, rinsed with water and air-dried. The strain gauges were bonded using cyanoacrylate cement (Super Bonder Loctite; Henkel Ltda., Itapevi, SP, Brazil) and connected to a data acquisition device (AD-S0500IP; Lynx, São Paulo, SP, Brazil). Each specimen was connected to another tooth outside of the analysis process to compensate temperature fluctuations owing to gauge electrical resistance or local environment (Santos-Filho *et al.* 2008).

The specimens were submitted to compressive loading at a constant speed of 0.5 mm min^{-1}, using an 8.0-mm steel sphere (Fig. 1) in a mechanical testing machine (EMIC DL 2000; EMIC, São José dos Pinhais, PR, Brazil) to a maximum limit of 150 *N* (Soares *et al.* 2008b). The data were transferred to a computer that used specific acquisition, signal transformation and data analysis software (AqDados 7.02 and AqAnalisys; Lynx).

Fracture resistance was tested using a universal testing machine (EMIC 2000DL), and the load was applied using a steel sphere (diameter 8.0 mm) to the long axis of the tooth with a cross head speed of 0.5 mm min^{-1}. This dimension of the sphere allowed the load to be applied directly and only on the tooth structure, minimizing the isolated effect of the material and emphasizing the complex restoration of tooth and restorative material. The force required (*N*) to cause fracture was recorded by a 10-Kn load cell hardwired to software (Tesc; EMIC), which was able to detect any sudden load drop during compression. The fracture specimens were evaluated under an optical stereomicroscope to determine the failure mode according to the location of the failure using the classification system proposed by Burke (1992): type I, involving a small portion of the coronal tooth structure; type II, involving coronal tooth structure and cohesive failure of the restoration; type III, root fractures with cohesive failure of the restoration, which can be restored in association with periodontal surgery; and type IV, severe root and crown fracture, requiring extraction of the tooth.

Statistical analysis was carried out with a statistical package (SPSS Inc, Chicago, IL, USA). Strain and fracture resistance test data were normally distributed (Kolmogorov–Smirnov test). Two-way ANOVA was performed to analyse the influence of the two factors in study (restorative material and irradiation) and their interactions on fracture resistance and strain for each cusp. *Post hoc* multiple comparisons were performed by using the Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). The failure mode was described by frequency.

Results

The fracture resistance test results are shown in Table 1. Two-way ANOVA revealed that the factor irradiation ($P = 0.017$) and the interaction between restorative material and irradiation ($P = 0.024$) were significant. The factor restorative material ($P = 0.767$) was not significant. In nonirradiated groups, teeth restored with amalgam had significantly lower fracture

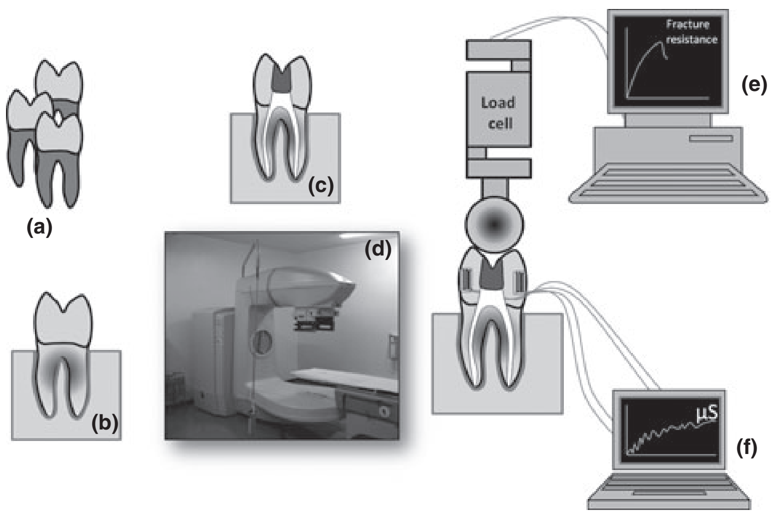

Figure 1 Schematic diagram of experiment tests: (a) tooth selection, (b) embedded tooth simulating periodontal ligament, (c) gamma radiation therapy, (d) endodontic treatment and direct restoration, (e) fracture strength test ($n = 10$), (f) strain gauge test (half of the samples per group were measured, $n = 5$).

Table 1 Mean values (SD) for experimental groups of fracture strength values (N)

Tooth condition	Nonirradiated	Tukey category*	Irradiated	Tukey category*
Sound	939.5 (293.5)	Aa	608.5(184.8)	Ba
Amalgam	674.4 (194.4)	Ab	664.3(195.3)	Aa
Resin composite	783.2 (232.4)	Aab	710.6(301.3)	Aa

*Tukey categories with same letters are not statistically significant from each other ($P < 0.05$). Capital letters were used to compare groups in the rows (radiotherapy factor) for each tooth condition separately, and lower-case letters were used to compare groups in the columns (tooth condition) for each irradiation condition separately.

Table 2 Failure modes distribution in experimental groups ($n = 10$)

Group	I	II	III	IV
Sound and nonirradiated teeth	9	–	–	1
Root filled, nonirradiated teeth restored with composite resin	6	2	2	–
Root filled, nonirradiated teeth restored with amalgam	3	2	3	2
Sound teeth and irradiated	9	–	–	1
Root filled, irradiated teeth restored with composite resin	2	1	2	5
Root filled, irradiated teeth restored with amalgam	4	2	3	1

I, fracture involving a small portion of the coronal tooth structure; II, fracture involving coronal tooth structure and cohesive failure of the restoration; III, root fracture with cohesive failure of the restoration; IV, severe root and crown fracture.

resistance mean values ($P = 0.041$) than intact teeth. That difference disappeared when teeth received the radiotherapy protocol.

The failure mode analysis (Table 2) indicated that sound teeth, whether irradiated or nonirradiated, had predominantly type I failures (90%). Teeth restored with amalgam had type I (30%) and type III failures (30%). The irradiated teeth restored with amalgam had type I (40%) and type III failures (30%). The teeth restored with resin composite had predominantly type I failures (60%). On the other hand, the teeth irradiated restored with resin composite had mostly type IV failures (50%).

The mean buccal cusp strain values of the groups are shown in Table 3. Two-way ANOVA revealed that the restorative material ($P < 0.001$), gamma radiation ($P < 0.001$) factors and the interaction between two factors ($P = 0.032$) were significant. In nonirradiated and irradiated groups, teeth restored with amalgam had significantly higher strain ($P = 0.012$) than sound

Table 3 Mean values of the microstrains (μS) and SD of the buccal cusps, and statistical categories defined by Tukey test ($n = 5$)

	Buccal cusp			
Restorative material	Nonirradiated	Tukey category*	Irradiated	Tukey category*
Sound	128.0 (27.6)	Aa	188.7 (31.6)	Aa
Amalgam	367.5 (118.5)	Ab	405.4 (116.1)	Bb
Resin composite	182.9 (85.4)	Aa	253.1 (109.5)	Aa

*Tukey categories with same letters are not statistically significant from each other ($P < 0.05$). Capital letters were used to compare groups in the rows (radiotherapy factor) for each tooth condition separately, and lower case letters were used to compare groups in the columns (tooth condition) for each irradiation condition separately.

teeth and teeth restored with composite resin. Similar strain means were found between sound teeth and teeth restored with composite resin, irrespective of gamma radiation. Gamma radiation increased the buccal cusp strain only for root treated teeth restored with amalgam.

The mean palatal cusp strain values of the groups are shown in Table 4. Two-way ANOVA revealed that the restorative material ($P = 0.023$), gamma radiation ($P = 0.016$) and the interaction between two factors ($P = 0.043$) were significant. In nonirradiated groups, teeth restored with amalgam had significantly higher strain ($P = 0.024$) than sound teeth restored and teeth restored with composite resin. Similar strain means were found between sound teeth and teeth restored with composite resin. In the irradiated groups, teeth restored with amalgam or composite resin had significantly higher strain ($P = 0.32$) than sound teeth. Gamma radiation increased the palatal cusp strain for all groups tested.

Table 4 Mean values of the microstrains (μS) and SD of the palatal cusps, and statistical categories defined by Tukey test ($n = 5$)

	Palatal cusp			
Restorative material	Nonirradiated	Tukey category*	Irradiated	Tukey category*
Sound	202.9 (83.4)	Aa	312.3 (164.3)	Ba
Amalgam	374.6 (133.0)	Ab	502.2 (211.7)	Bb
Resin composite	254.4 (68.0)	Aa	489.5 (40.0)	Bb

*Tukey categories with same letters are not statistically significant from each other ($P < 0.05$). Capital letters were used to compare groups in the vertical lines (restorative material), and lower case letters were used to compare groups in the horizontal lines (irradiation factor).

Discussion

The null hypothesis was rejected as the gamma radiation influenced the biomechanical behaviour of human premolars. Radiotherapy is an inevitable component of contemporary cancer management therapy, which includes irradiation of the tumour mass with ionizing radiation (Aggarwal 2009). The present study revealed that the radiotherapy contributed to tooth weakness, because the values of fracture resistance of sound irradiated teeth were significantly lower and strain of cusps were significantly higher than the nonirradiated ones.

The ionizing radiation causes tissue injury in two ways: direct and indirect (Chistiakov *et al.* 2008, Sarkaria & Bristow 2008). As a direct mechanism, the ionizing radiation generates electron loss as well as electron gain centres through ejection of electrons and capture of ejected electrons, respectively. There is a direct alteration in biologic molecules, and approximately one-third of biologic effects results from a direct effect. The indirect mechanism involves the reaction of the target tissue with free radicals produced by the action of radiation on water. The majority of radiation induced biologic damage results from indirect effects (Chistiakov *et al.* 2008, Sarkaria & Bristow 2008).

Xerostomia is a common and serious side effect of radiotherapy for head and neck cancer and often enhances caries activity (Franzén *et al.* 1992). The treatment includes salivary substitutes and restoration of the carious lesions (Ritchie *et al.* 1985). It has been shown that irradiation of protein leads to changes in their secondary and tertiary structures. The formation of free radicals, from the reaction with the water molecules before the absorption of ionizing rays (Pioch *et al.* 1992), promotes the denaturing of the organic components of teeth (Baker 1982, Pioch *et al.* 1992, Soares *et al.* 2010). It may also affect the existing resin–dentine interface by affecting the hybrid layer (Aggarwal 2009). Moreover, radiotherapy significantly reduces the intrinsic resistance of enamel and dentine with the most damaging effect on the proteic components (Cheung *et al.* 1990, Soares *et al.* 2010), decreasing the stability of dentine (Pioch *et al.* 1992, Soares *et al.* 2010).

The damage to collagen fibres promoted by irradiation could result in impaired bond strength between composite and dentine (Cheung *et al.* 1990). Moreover, it has been reported that the apatite crystals of tooth hard tissues incorporate sodium, carbonate and magnesium by entrapment during their formation (Jansma *et al.* 1990). When irradiated, these point defects could be mobilized from the surface layer of crystals, removing the entrapped ions and modifying the structure of the crystals, thus potentially interfering with the adhesion (Biscaro *et al.* 2009). This hypothesis was confirmed by this study, because the radiotherapy influenced significantly the adhesion of the composite resin. Samples restored with composite resin or amalgam presented similar fracture resistance with a drastic change in the fracture mode for the group restored with composite resin. The bonding condition was compromised and the failure progressed through the interface. Previous studies suggested that restorations with composite resins might show reduced life expectancy in irradiated patients (Gernhardt *et al.* 2001). However, little is known about the effects of irradiation on composite restorations.

A study reporting a large number of nuclear alterations in pulp fibroblasts and odontoblasts after Co60-fractioned radiotherapy (Vier-Pelisser *et al.* 2007) showed that, comparing the immediate and delayed effects of radiotherapy, the immediate alterations were more obvious than those after 30 days. This suggests that the immediate results of this study could be transitory. Future studies should be performed to evaluate the long-term effects to obtain information that could be extrapolated to clinical practice, helping practitioners understand what could be expected regarding to the bonding conditions, fracture resistance and tooth strain when patients are submitted to head and neck radiotherapy.

The strain gauge test showed that the buccal cusp had more strain than the palatal, which can be explained by the configuration of the premolar and by the dimension and position of the loading sphere during the test. In general, nonirradiated teeth had more strain when they were restored with amalgam than with composite resin. These findings confirm the results from previous studies (Assif *et al.* 1990, Medige *et al.* 1995, Soares *et al.* 2008b). This fact can be related to the bonding promoted by the adhesive system between composite resin and tooth structures (Assif *et al.* 1990, Medige *et al.* 1995, Soares *et al.* 2008b). The irradiation protocol may have influenced the adhesion of composite resin, because the irradiation increased the strain when the teeth were restored with composite resin. On the other hand, the gamma radiation increased the buccal cusp strain for teeth restored with amalgam, revealing that additional alterations were produced not only on the adhesive procedure, but also on the tooth tissues. The gamma radiation increased the palatal buccal strain for all groups tested. This difference between buccal and palatal cusps may be explained because the palatal functional cusp is stressed more during occlusal load. Evaluating the effect of gamma irradiation on enamel and dentine, Soares *et al.* (2010), showed that irradiated dentine had microcracks and collagen degradation, whilst irradiated enamel had irregularities in the interprismatic structure resulting in a melted surface. These alterations were explained by a disarrangement of the crystalline portion of enamel (Jansma *et al.* 1988) and by a denaturation of the organic matrix caused by radiolysis (Pioch *et al.* 1992), which promoted alterations in crystalline organization and of protein interprismatic links (Baker 1982). However, in the present study, the fracture pattern of sound teeth was the same either for irradiated and nonirradiated sound teeth. This suggests a similar stress distribution pattern, but the weaker interprismatic substance and the protein degradation promote a rapid propagation of stress across the irradiated specimens (Soares *et al.* 2010), causing it to fail under a lower load. The denaturation of the organic matrix caused by radiolysis would reduce the physical anchorage between enamel and dentine (Pioch *et al.* 1992). Another important observation in the present study was that samples restored with composite resin had a drastic change in fracture mode, demonstrating that irradiation affected the adhesive integration of the collagen network.

The findings of this study are of fundamental importance for teeth that were restored; however, additional research and clinical studies are required. There are few data in the literature evaluating the correlation between radiotherapy and the biomechanical behaviour of teeth.

Conclusion

Within the limitations of this laboratory study, the following conclusions were drawn:

1. Gamma irradiation reduced significantly the fracture resistance of sound teeth.
2. Tooth strain was, in general, higher when teeth were restored with amalgam than with composite resin regardless of their exposure to gamma radiation.
3. Nonirradiated teeth restored with composite had no significant increase in strain compared with sound teeth.
4. Fracture mode and teeth strain results suggest that composite resin gave better biomechanical behaviour than amalgam for restoring root filled teeth, whether or not they were submitted to radiotherapy protocols.

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