Original Article

Impact of gamma radiation on marginal adaptation of nanohybrid composite and composition of dental hard tissues – Scanning electron microscopy and X-ray diffraction analysis: An *in vitro* pilot study

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Abstract

Aims: This pilot study aimed to compare the marginal adaptation of composite resin at the tooth-restoration interface, before and after radiation.

Subjects and Methods: Fifteen extracted premolars were divided into 2 experimental groups (based on the timing of irradiation) and 1 control group of 5 teeth each. In Group I (control group), teeth were restored but not exposed to radiation at any stage, Group II: teeth were irradiated before cavity preparation and restoration, and Group III: after cavity preparation and restoration employing selective etch technique, teeth were exposed to radiation. The samples were then sectioned buccolingually to analyze the extent of the marginal gap under scanning electron microscopy and compositional alteration of dental hard tissues by X-ray diffraction study. The data collected were analyzed statistically.

Statistical Analysis Used: The statistical software used was IBM SPSS version 23 New York, USA, and analysis was done using two-way ANOVA followed by Turkey's *post hoc* test, this difference in the mean marginal gap between all three groups was nonsignificant ($P \geq 0.05$).

Results: In the control group (Group I), a minimum gap (4.203 μm ± 0.533) was observed at the tooth-restoration interface, indicating the highest level of adaptation as compared to Group II (5.816 μm ± 0.762) and Group III (4.862 μm ± 1.018). This suggests that radiation adversely affected the bonding between composite materials and both enamel and dentin, attributed to the alterations induced by radiotherapy in the chemical, physical, and morphological properties of both tooth structure and composite resin.

Conclusions: Ionizing radiations adversely affect the bonding between enamel, dentin, and composite resin. Hence, restorative procedures should be performed before undergoing radiotherapy.

Keywords: Composite resin; irradiation; marginal adaptation; scanning electron microscopy

INTRODUCTION

Oral cancer is a growing concern globally, ranking sixth among cancers worldwide. India bears a hefty burden, shockingly accounting for nearly one-third of all reported

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cases worldwide. In our country, tragically about 70% of cases are detected at an advanced stage (Stage III–IV). Unfortunately, this results in extremely low chances of cure nearly nonexistent, only about 20% survive for 5 years postdiagnosis.[1] Oral cancer starts as a small, unexplained sore or growth on the lips, cheeks, tongue, and palate. Without timely detection, it can spread to the base of the mouth and oropharynx.[2]

For over half of patients, radiotherapy is a common treatment either sole or in combination with chemotherapy or surgery. Radiation doses typically range from 40 to 70 Gy, occasionally reaching up to 80 Gy.[3] Radiotherapy can induce clinical challenges in the oral cavity and salivary glands, encompassing issues such as hyposalivation (irreversible dry mouth increasing caries risk), mucositis, taste loss (typically reversible), and trismus.[4] In addition, it can weaken the mechanical strength of enamel and dentin, potentially resulting in the development of cracks. Hence, it is crucial to prioritize preventive measures, especially in high-dose radiation cases, to protect oral health and dental integrity.[5]

Dental decay commonly occurs in pits, fissures, and proximal surfaces. However, radiotherapy changes saliva production, making decay more likely at cuspal inclines and near the gumline. This shift increases decay prevalence in cervical areas, often necessitating specific restorative treatments.[6] Adhesive restorative materials like composites are preferred over metallic. This preference arises because of the high atomic mass number of metallic materials, raising the risk of radio-mucositis. In addition, metal compounds can contribute to secondary radiation backscattering, worsening the condition. Therefore, nonmetallic adhesive materials help reduce these complications, promoting the well-being of patients undergoing radiotherapy.[7]

Marginal adaptation in adhesive restorations is paramount. The poor marginal adaptation can cause discoloration, sensitivity, and secondary caries, necessitating restoration replacement or repair.[8]

SUBJECTS AND METHODS

In this pilot study, 15 freshly extracted premolars were collected and divided randomly into three groups. One group served as the control, whereas the remaining two groups were experimental, each containing 5 teeth.

Sample preparation

Class V cavities of uniform dimensions, i.e., 3 mm × 2 mm × 1.5 mm, were prepared on the buccal surface of each tooth. The coronal portion extended into the enamel and the apical into the cementum. The samples were restored with composite resin, according to the radiation stage.

Group I (control group) ($n = 5$) – Cavities were prepared and restored but not exposed to radiation at any stage

Group II ($n = 5$) – Teeth were irradiated first and then cavities were prepared and restored

Group III ($n = 5$) – First cavities were prepared and restored and then teeth were irradiated.

Restorative protocol

Phosphoric acid gel (Tetric N-Etch) was applied on the enamel only (Selective-Etch Technique) of the prepared cavity using a fine needle for 15 s and then rinsed thoroughly with water for 20 s. The cavity was then dried with blotting paper to leave the cavity slightly moistened. A layer of bonding agent (Tetric N-bond universal, i.e. self-etch adhesive) was applied to moist, etched, and prepared cavity surfaces carefully with the help of an applicator tip. The excess bonding agent was evaporated with the help of a gentle air stream, followed by light polymerization for 10 s, as instructed by the manufacturer. The increments of composite resin were placed into the prepared cavity using a Teflon-coated instrument and polymerized by light for 10 s.

Radiation protocol

Radiation therapy was performed using a cobalt-60 teletherapy machine (Homi Bhabha Cancer Hospital and Research Centre, Sangrur), as per the therapeutic dose for head-and-neck cancer treatment. The samples were irradiated to 70 Gy gamma radiation, in fractionated dose (2 Gy/5 days/week), delivered in daily sessions, lasting for 7 weeks.

The samples were then stored in 0.9% normal saline till further use. To assess any gaps at the tooth-restoration interface and compositional alteration, the samples were longitudinally sectioned from the center buccolingually using a diamond-coated saw. One part of the sectioned sample was gold sputtered and examined, under a scanning electron microscope. This gap was measured in micrometers. The other part of the sectioned sample was studied with X-ray diffraction (XRD) for compositional changes.

Statistical analysis

The statistical software used for analysis was IBM SPSS version 23 New York, USA. Numerical data were explored and represented by mean and standard deviation values. A two-way ANOVA test was used to explore the significance of the marginal gap between all three groups followed by Tukey's *post hoc* test.

RESULTS

In Group I, a minimum gap was observed at the tooth-restoration interface with a mean (4.203 µm ± 0.533), indicating the highest level of adaptation, compared to the experimental groups (II, and III) with a mean of 5.816 μm ± 0.762 and 4.862 μm ± 1.018), respectively [Graphs 1 and 2]. This difference in marginal adaptation was statistically insignificant ($P \geq 0.05$) [Tables 1 and 2].

X-ray diffraction study

It showed equal distribution of elements in the control group (Group 1) with minor peaks corresponding to Sr_2, Pb (H2Po4) 2, Ca, and Pb, suppressed and missing peaks in Group(s) II and III, respectively [Graph 3].

DISCUSSION

Cancer, marked by cells growing out of control and invading nearby tissue, is a leading cause of death.[9] The primary goal of treatment is to eliminate cancer cells while preserving healthy tissue, either solely by radiotherapy or along with chemotherapy or surgery.[3]

According to Bozan *et al*.,[10] radiotherapy has the potential to demineralize tooth hard tissues, leading to enamel and dentin breakdown, which in turn reduces microhardness and increases surface roughness.[11] Moreover, a decrease in salivary pH, along with its diminished ability to remineralize enamel and inadequate oral care, can result in radiation-induced caries that spreads circumferentially around the tooth.[12]

Lesions typically emerge 4 weeks after radiotherapy, targeting unusual tooth areas such as the lingual surface, incisal edges, and cusp tips. Clinically, three distinct varieties have been recognized. The most frequent (Type 1) affects the cervical aspect extending along the CEJ, often leading to crown amputation as the lesion develops circumferentially. In the second pattern (Type 2), demineralization occurs across all dental surfaces, often accompanied by generalized degradation and worn biting and cutting surfaces. The third and rarest pattern (Type 3) is that dentin exhibits color changes, resulting in a dark brown/black crown, sometimes accompanied by visible occlusal and incisal wear. It is worth noting that one individual may display multiple patterns and more importantly, even teeth present away from the radiotherapy field can be affected.[13]

Table 1: Mean and standard deviation of marginal gap for each group and comparison of mean marginal gaps in study groups by two-way ANOVA test

Groups	*n*	Mean±SD	95% CI mean	
			Upper	Lower
Group 1	5	4.204±0.533	4.866	3.541
Group 2	5	5.186±0.762	6.132	4.241
Group 3	5	4.863±1.018	6.127	3.598

Source	Sum of squares	Df	Mean square	*F*	*P*
Between groups	2.535	2	1.25	1.98	0.180
Within groups	7.607	12	1.25		

Inference: The *P* value > 0.05, indicating no statistically significant difference in the mean marginal gap between the study groups. SD: Standard deviation, CI: Confidence interval

Table 2: Pair-wise comparison with *post hoc* analysis (Tukey test)

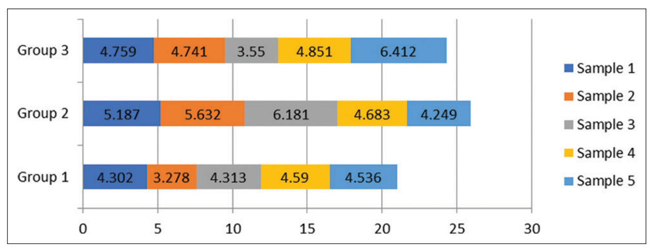
	Mean difference	SE	*t*	*P*
Group 1				
Group 2	−0.983	0.512	−1.921	0.339
Group 3	−0.659	0.512	−1.288	0.701
Group 2				
Group 3	0.324	0.512	0.633	0.968

Inference: The pair-wise comparison of the study groups revealed a *P* value > 0.05 for each comparison, indicating no statistically significant difference in the mean marginal gap between the study groups. SE: Standard error

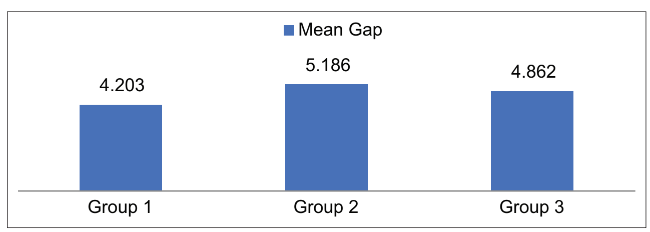
In vitro study

This study to assess the marginal adaptation of composite resin before and after radiation using scanning electron microscopy (SEM) was conducted *in vitro* because it is practical to conduct and can be easily duplicated.[14]

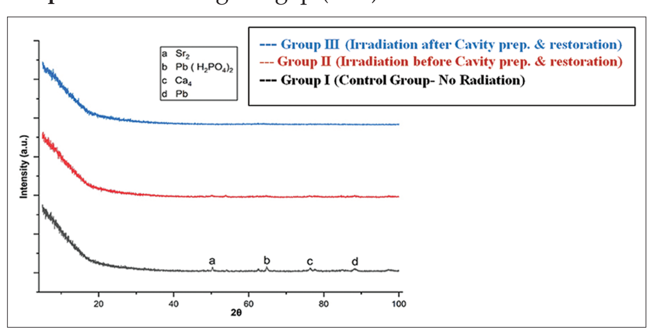
Previous research has demonstrated that patients undergoing radiotherapy are highly susceptible to experiencing dental caries in the cervical areas of the teeth. Therefore, Class V cavities were prepared for



Graph 1: Marginal gap (mm) in samples of various groups



Graph 2: Mean marginal gap (mm)



Graph 3: X-ray diffraction spectra showing characteristic patterns for each study group

this study. Moreover, adhesive restorative materials like composites are preferred over metallic, due to the risk of radio-mucositis.[7]

Cavity margins involving enamel were etched by selective etching technique as Buonocore demonstrated that the incorporation of acid-etched enamel margins significantly diminishes microleakage in composite resins.[15] Hegde *et al*.[16] showed that self-etch adhesive systems used with the selective etch technique result in minimum microleakage.

The selective-etch method overcomes a key limitation of the self-etch technique by ensuring adequate etching of mineralized enamel. Consequently, it optimizes the overall bonding process, offering improved outcomes in dental restorations. Talan *et al*.[17] recommended etching of enamel surface before application of any adhesive system to achieve adequate bond strength. The 7th-generation bonding agent, termed a "multimode" adhesive system, is versatile, allowing for use in self-etch, etch and rinse, or selective-etch techniques.[18] Jamadar *et al*.[19] found that 7th-generation bonding agents show superior bond strength than 6th generation. Composite resin was placed in increments as it provides minimum microleakage as compared to bulk placement.[20]

Samples in Group I were not exposed to radiation at any stage.

Group II samples illustrate patient's clinical condition, mainly oral squamous cell carcinoma (OSCC), representing 84%–97% of cases. While radiotherapy effectively treats this condition, it can lead to complications such as xerostomia, radiation caries, and osteoradionecrosis. Thus, specialized dental care is imperative.[1]

Group III samples comprise OSCC patients advised for radiotherapy. Before treatment, physicians commonly recommend dental interventions, often involving the replacement of metal-based restorations like amalgam with tooth-colored polymer-based materials due to their favorable clinical performance.[21]

Both experimental groups were subjected to a radiation regimen of 70 Gy, using gamma radiation from a cobalt irradiation unit (teletherapy machine, Homi Bhabha Cancer Hospital-TMC Sangrur). According to therapeutic fractioned dose, over a span of 7 weeks, intending to replicate clinical conditions and respect the 5 R's (repair, redistribution, reoxygenation, regeneration, and radiosensitivity). Although none of the 5 R's could happen as its *in vitro* study.[22]

The samples were then longitudinally sectioned from the center of the cavity buccolingually, using a diamond-coated saw (Contiene: Disco de Diamante 0.20 mm), as recommended by Fränzel *et al*.[23]

SEM analysis yields observational data measuring the marginal gap in micro gaps (µm) at the tooth-restoration interface, through its exceptional magnification and resolution capabilities, thereby yielding clear images [Figure 1]. SEM analysis demonstrated that superior marginal adaptation was attained when teeth underwent irradiation subsequent to cavity preparation and restoration, suggesting that radiation adversely affected the bonding between composite materials and both enamel and dentin, attributed to the alterations induced by radiotherapy in the chemical, physical, and morphological properties of both tooth structure and composite restorations.

Other studies corroborating the findings of this *in vitro* study are:

Naves *et al*.[7] concluded that the timing of restorative procedures concerning radiotherapy influences the bonding capacity of both enamel and dentin. Gamma radiation significantly reduces bond strength to human enamel and dentin by impairing the formation of the hybrid layer and increasing the occurrence of cohesive failures within the dental substrate when the adhesive restorative procedure is performed after radiotherapy. Conversely, when the restoration is conducted before irradiation, no significant alteration in bond strength is observed.

According to Tikku *et al*.,[24] the activation of matrix metalloproteinases in the dentin matrix as a result of radiotherapy could contribute to debonding at the

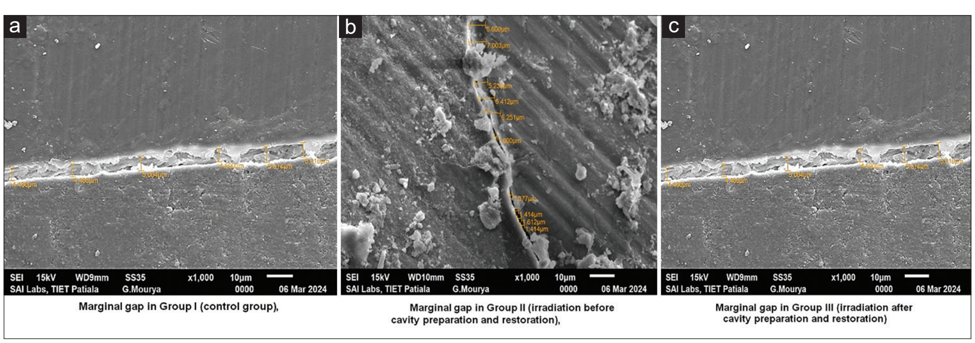


Figure 1: (a) Marginal gap in Group I (control group), (b) Marginal gap in Group II (irradiation before cavity preparation and restoration), (c) Marginal gap in Group III (irradiation after cavity preparation and restoration)

tooth-restoration interface, leading to increased marginal gaps after radiotherapy. In addition, radiotherapy negatively impacts collagen fibers, diminishing the bond strength between dentin and composite, and adversely affecting marginal adaptation. Consequently, concluding that the restoration should be undertaken before radiotherapy in patients with head-and-neck cancer.

Velo *et al*.[25] radiation interacts with the high water content in dentin, resulting in radiolysis and the production of H^+ ions and OH^- ions. These ions then combine with other ions to form new compounds, with the resulting free radicals lingering in dentin for an extended duration. Furthermore, radiation leads to a reduction in "C" ions and "Ca/P" weight and triggers the formation of a secondary nonapatitic calcium (Ca) phosphate phase which increases the susceptibility of hydroxyapatite to degradation. In addition, the presence of amorphous structures and improper crystalline arrangements contributes to crack formation. Collectively, these alterations impede the bonding of restorative materials to dentin.

Reichmanis *et al*.[26] found that gamma radiations involved in radiotherapy have high energy which leads to excitation and ionization in polymers generating free radicals that can alter the physical, chemical, and mechanical properties of the polymers. This change occurs simultaneously with polymer degradation.

Troconis *et al*.[27] and Gupta *et al.*[28] stated that the ionizing radiation did not hinder the bonding of adhesive systems when the bonding procedure was conducted before radiotherapy. Therefore, restorative procedures should be carried out before radiotherapy.

However, there are other studies that are in contrast to the findings of this *in vitro* study:

Hu *et al*.[29] showed that damage to the collagen network caused by irradiation does not disrupt the formation of the hybrid layer and therefore does not hinder the adhesive capacity between dentin and composite resin materials.

Silva *et al*.[30] stated that the increased failure of dental restorations seems to be linked to the presence of demineralized dentin adjacent to restorative materials, resembling secondary and residual caries, as it can compromise the adhesion of restorative materials. Therefore, concluded that demineralization is the cause of restoration failure, rather than radiation.

X-ray diffraction analysis

XRD spectra of samples showed that there is an equal distribution of elements in the control group with minor peaks corresponding to Sr_2, $Pb(H_2PO_4)_2$, Ca, and Pb. Strontium (Sr_2) is essential to prevent demineralization, whereas lead hydrogen phosphate [$Pb(H_2PO_4)_2$] helps in improving remineralization, thereby protecting enamel, Ca is vital for tooth structure, and it protects and strengthens enamel, and lead (Pb) is found as trace element but its absence may alter the development of dental hard tissue.

Radiation has a deleterious effect on the elemental composition of tooth as these peaks were suppressed in Group II and absent in Group III. Qing *et al*. also showed that radiation significantly decreased the mineral content of enamel and dentin. Moreover, radiation causes cracks and obliteration of dentinal tubules with the destruction of the collagen fibril network, leading to dry and friable dentin.[31-33]

Limitations of the study

It's crucial to recognize the need for a large sample size to validate the results of this pilot study. In addition, accurately replicating the complexity of *in vivo* conditions poses a significant challenge. Clinical exposure to these radiations results in adverse effects such as hyposalivation, decreased saliva buffering capacity, altered salivary electrolyte levels, and significant changes in oral flora. These factors collectively diminish the remineralizing capacity of saliva, leading to radiation caries. Therefore, we recommend further validation through *in vivo* studies. Furthermore, teeth from radiation-exposed patients are not available, as they are extracted before radiotherapy, so any structural and compositional changes due to the altered oral environment from radiation cannot be studied.

CONCLUSIONS

- The radiation therapeutic dose for head-and-neck cancer patients adversely affects bonding between polymer resin and dental hard tissue
- Restorative procedures should be carried out before the initiation of radiotherapy.

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Conflicts of interest

There are no conflicts of interest.

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