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Original Article

Effect of the cavity disinfectant containing chitosan on dentin bonding strength after radiotherapy

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Abstract

Introduction: This study aimed to compare the bond strength of teeth treated with radiotherapy with two cavity disinfectants (Chlorhexidine gluconate, a chitosan-containing agent).

Methodology: Eighteen newly extracted, non-carious human third molar teeth were used. The teeth were randomly divided into two main groups, treated and/or non-treated with radiotherapy, then separated into three subgroups for disinfectant agent application (CHX, chitosan, control). A total dose of 70.2 Gy was given over 39 days using a linear radiation accelerator for radiotherapy. After applying the cavity disinfectant, the teeth were restored with composite resin to obtain the stick. All bond strength values from sticks were measured using a universal testing machine. The data were analyzed with ANOVA and Chi-square test at a $p < 0.05$.

Results: Among all groups, Group CH, with no radiotherapy application and containing a chitosan-disinfectant agent, showed the highest bond strength (44.7 ± 8.2). In contrast, Group RC with radiotherapy and disinfectant-free showed the lowest bond strength value (29.1 ± 3.5). The highest bond strength values were obtained after applying the chitosan-containing agent in all groups.

Conclusions: Radiotherapy application had a negative effect, while the use of disinfectant agents had a positive effect on the bond strength.

Key words: Radiotherapy; chitosan; chlorhexidine; microtensile.

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Introduction

Radiotherapy is frequently used in the treatment of head and neck cancers, which is of great importance in dentistry [1]. Radiotherapy application is technically complicated due to the vital structures in the head and neck [2]. It is known that changes in the mechanical properties of dental hard tissues occur due to hyposalivation caused by radiotherapy on the tooth [3]. In addition, radiotherapy damages tooth hard tissues such as enamel and dentin, resulting in the formation of caries, deterioration of the dentin-enamel junction, loss of the bonding function of the hybrid layer with damage to collagen fibrils, and decreased bond strength [4].

Matrix metalloproteinases (MMPs) are a calcium-dependent homologous enzyme family that can degrade the extracellular matrix and basement membrane components and contains zinc in their active site [5]. The degradation occurs at the resin-dentin interface with the activation of MMP [6]. Radiotherapy causes damage to collagen fibrils and leads to a decrease in bond stability, tensile strength, abrasion resistance, and the hardness of dentin [7]. In addition, as a result of damage to the chemical structure of dentin, the bond strength of the adhesive systems deteriorates, and restorations fail [8].

Some studies have shown that disinfectants with an MMP inhibitory effect applied to the dentin before restoration increase the adhesion by maintaining the stability of the hybrid layer, thus prolonging the life of the restorations [9]. Chlorhexidine gluconate (CHX) is the most preferred solution used for this purpose [10]. However, many studies have shown that CHX delays the degradation process of the hybrid layer containing resin-dentin structures [11,12].

In recent years, researchers have focused on using chitosan that a hydrophilic biopolymer containing many free hydroxyl and amino groups [13]. It is a non-toxic, antifungal, antioxidant with antibacterial effects. Also, chitosan has been shown to form a reinforcing matrix in dentin and improve collagen's biological and mechanical properties [14]. Moreover, chitosan is an antimicrobial agent that can reduce the degradation of the collagen matrix caused by MMPs activated by radiotherapy [15]. For this reason, we aimed to develop a chitosan-containing cavity disinfectant and compare it with CHX in our study.

In the available literature, there is no study on chitosan-containing disinfectants after radiotherapy application. This study evaluated the effect of the disinfectant application containing CHX and chitosan after radiotherapy on bond strength. This disinfectant aims to reduce the frequency of replacement of dental restorations. The null hypotheses were as follows:

i) Disinfectant containing chitosan would not reduce bond strength.

ii) Radiotherapy would not enhance bond strength.

Methodology

Sample Size

To determine the number of samples before starting the study, we performed a power analysis using the software G*Power 3.1.9.4. For the SBS analysis, the minimum sample size was determined to be 72 for an effect size of 0.63, 95% power (1-β), and a 5% (α) confidence interval.

Study Groups

1. CHX: Chlorhexidine
2. CH: Disenfectant containing chitosan
3. C: No radiotherapy, no disinfectant application
4. R + CHX: Radiotherapy and application of Chlorhexidine
5. R + CH: Radiotherapy and application of disinfectant containing chitosan
6. R + C: No disinfectant application

Radiotherapy application

X-ray computed tomography (CT) was used to determine the radiation dose to be applied to the teeth. Considering the dose of the patients who received radiotherapy to the head and neck, the dose to be applied to the extracted teeth (1.8 Gy × 39 fractional doses) was determined as 70.2 Gy 3D conformal planning technique was used as treatment presentation [16]. During irradiation, the teeth were covered with a rubber material to simulate extraoral tissues. Teeth were kept in distilled water, and the water changed daily.

Preparation of cavity disinfectant with chitosan

0.2 g chitosan (Sigma Aldrich Chemical, St. Louis, MO, USA) was added to 100 mL of 1% acetic acid by weighing it on a precision balance (Sartorius, TE 214 S). The solution was mixed with a magnetic stirrer to dissolve, and the cavity disinfectant was prepared.

Preparation of samples

The enamel tissue on the occlusal surface of the teeth was removed entirely using a diamond separator (Isomer 1000, Buehler Ltd., Lake Bluff, IL, USA) under water cooling to expose the dentin tissue. Sanding and polishing the surfaces of the samples were done with 600, 800, and 1000 grit silicon carbide abrasive papers (3M ESPE, St. Paul, MN, USA) in a polishing machine (LaboPol-5, Struers, Denmark). Thus, a standard smear layer was formed on the exposed dentin surfaces. The cavity disinfectants containing 2% CHX (BISCO's Cavity Cleanser, Bisco, Schaumburg, Illinois, United States) and 0.2% chitosan were applied to the dentin surface with the help of a cotton ball for 60 seconds. Clearfil Universal Bond (Kuraray, Japan) was used according to the manufacturer's instructions. Then, a 4 mm thick composite (G-ænial Posterior, GC, Japan) was placed with a 2 mm layering technique. Each layer was polymerized with an LED (Valo Led, Ultradent) light device for 20 seconds. The specimens were thermocycling for 5000 cycles between 5 and 55 °C (SD Mechatronik Thermocycler, SD Mechatronik GMBH, Westerham, Germany). The stick (1 mm^2) was obtained from dentin-composite samples with a machine (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). The thickness of the specimens was measured using a digital caliper (Mitutoyo, Tokyo, Japan). The sticks were fixed with cyanoacrylate glue (Zapit; DAVA, Corona, CA, USA). A universal testing machine (BISCO; Schaumburg, IL, USA) was used for the micro tensile test at a crosshead speed of 1 mm/minute until failure. Values were recorded in Newtons (N) and converted to MPa.

Failure Analysis

The maximum force (N) at failure was recorded. Considering the bonded area on the tooth surface, we calculated the SBS in MPa. The fractured were analyzed under an optical microscope (Leica Microsystems, Wetzlar, Germany) at 25x magnification to determine the fractured status. The type of failure was categorized as follows: (a) adhesive failure (those which occurred between the composite resin and the dentin surface), (b) cohesive failure (those which occurred within the resin composite), and (c) mixed failure (adhesive and cohesive fracture of the material).

Statistical analysis

Analysis was performed using the SPSS 22.0 program (IBM SPSS Statistics 22.0, SPSS Inc., an IBM Co., Armonk, New York, USA). Before statistical

Table 1. Means and standard deviations of the MPa values (MPa) for different groups.

	Group CHX	Group CH	Group C	Group RCHX	Group RCH	Group RC
MPa Mean ± SD	39.4 ± 8.2[a]	44.7 ± 8.2[ac]	34.5 ± 3.0[a]	33.2 ± 2.8[ab]	40.3 ± 6.1[ac]	29.1 ± 3.5[b]

*Different letters within lines indicate statistically significant differences. Lower cases represent linear differences.

analysis, we analyzed the normality of the data with the Shapiro-Wilk test. One-way ANOVA with a post-hoc Tukey test was used to compare differences between more than two independent groups. We used the paired t-test to compare differences between pairs of values. The Chi-square test was applied to compare the failure analysis. Finally, the statistical significance level (p) was < 0.05.

Results

Findings of the Bond Strength (Mpa) Test and Failure

The findings indicate that there is a significant difference between Group RC (28.02 ± 6.81) ($p < 0.05$) and Groups RCH (40.3 ± 6.1), CH (44.7 ± 8.2), C (34.5 ± 3.0), and CHX (39.4 ± 8.2); these four groups are similar to each other ($p > 0.05$). There are no significant differences among Groups CH (44.7 ± 8.2), RCH (40.3 ± 6.1), and the control group ($p > 0.05$). Table 1 shows the MPa values.

The results of the failure status examination revealed that the adhesive modes of failure are the most predominant failure modes in all groups. The failures are seen in all groups (Table 2).

Discussion

Our hypotheses were accepted: i) disinfectant containing chitosan would not reduce bond strength, and ii) radiotherapy would not enhance bond strength.

In the available literature, there are many studies on how the changes in dental hard tissues caused by radiotherapy applications affect the prognosis of restorations to be made after radiotherapy [17,18]. Studies show differences in vivo and in vitro regarding materials used and radiation dose; however, there is no clear result. Many researchers reported that radiotherapy causes some harmful effects on the physical, chemical, and histological structures of the teeth [19], which will negatively affect the restoration to be made [20]. However, contrary to other opinions, some researchers have argued that it does not cause harmful effects on dental hard tissues [21] and does not change the bond strength [22].

Mellara *et al*. [23] revealed that at doses higher than 30 Gy, damage in enamel's morphological structure and dentin was initiated. Rodrigues *et al.* [24] studied the biomechanical and dentin bonding properties after 72 Gy radiotherapy evaluated its strength and determined that mineral loss and irregularities in the dentin structure negatively affected the dentin bond strength. On the other hand, Markitziu *et al.* [25] reported that 72 Gy radiotherapy did not change the enamel microhardness values. According to the results from these studies, it can be stated that the effect of radiotherapy on teeth varied according to the applied radiation dose. Moreover, all results showed that bond strength values were negatively affected by radiotherapy. In our study, it was determined that the bond strength values in the groups that received radiotherapy were low, and it can be stated that radiotherapy caused an adverse change in the dentin structure.

As mentioned earlier, irradiation of dentin with radiotherapy and the presence of weak acids in bonding systems cause the activation of MMPs [26]. Cunha *et al.* [27] reported that MMP-9 proforms and active forms were more potent, investigating the effects of radiotherapy on endogenous enzymatic activity in dentin. Similarly, many researchers emphasized that endogenous collagenolytic enzymes, MMP, and cysteine cathepsins are responsible for the time-dependent degradation of hybrid layer collagen. MMPs are enzymes that can degrade hybrid layer collagen and reduce the life of adhesive restorations. The restoration durability time can be prolonged by inhibiting these enzymes [28].

Many researchers reported that CHX inhibited MMP activity and increased bond strength by reducing the rapid degradation of the hybrid layer [29]. It has been demonstrated that 2% CHX administration, known to have a broad-spectrum MMP inhibitory effect, significantly improved the integrity of the hybrid layer at a six-month clinical follow-up [30]. Saffarpour *et al.* [31] used CHX as an MMP inhibitor on the bond strength and found that CHX significantly increased the

Table 2. Frequency of types of bond failure for each group (%).

	Group CHX	Group CH	Group C	Group RCHX	Group RCH	Group RC
Adhesive	4/12 (33.3%)	3/12 (25%)	5/12 (41.6%)	7/12 (58.3%)	4/12 (33.3%)	9/12 (75%)
Cohesive	5/12 (41.6%)	5/12 (41.6%)	5/12 (41.6%)	1/12 (8.3%)	5/12 (41.6%)	1/12 (8.3%)
Mixed	3/12 (25%)	4/12 (33.3%)	2/12 (16.6%)	4/12 (33.3%)	3/12 (25%)	2/12 (16.6%)

bond strength. Soares *et al.* [32] suggested that mouthwash with CHX partially prevented damage to the mechanical properties of radiotherapy-treated dentin. Abdalla *et al.* [33] reported a decrease in enamel and dentin microhardness after radiotherapy, and CHX could be protective against hardness reduction and demineralization. This study determined that the application of CHX to teeth that underwent radiotherapy increased the bond strength values.

Chitosan is an organic biopolymer that can reduce the collagen matrix degradation caused by MMPs [34]. Daood *et al.* [35], in their study investigating demineralized dentin collagen fibrils and the resin-dentin junction's morphological and chemical changes, reported the addition of chitosan and riboflavin to the adhesive systems protected the demineralized dentin tissue and the hydrolytic and collagenolytic. Kong *et al.* [36] investigated the MMP-2 and MMP-9 inhibition activities of carboxymethyl-chitin and carboxymethyl-chitosan, which are water-soluble chitosan derivatives, and reported that they had effective results in MMP inhibition. Baena *et al*. [37] found that chitosan application decreased MMP activities, and resin-dentin bond strength increased. Paschoini *et al.* [38] evaluated the resin dentin interface bond strength of the total-etch Adper Single Bond and self-etch adhesive system Clearfil SE Bond after chitosan application to dentin. It has been reported that chitosan treatment preserves the bond strength, and no statistically significant difference was observed in dentin adhesion between adhesive systems. Lopes *et al.* [39] found that radiotherapy changed the structure of collagen and 0.2% chitosan increased dentin collagen resistance. It was determined that applying a chitosan-containing agent to the teeth, whether received or not radiotherapy, significantly increased the bond strength values in this study.

Walter *et al.* [40] found that hydrophilic monomers have a significantly stronger chemical bond to hydroxyapatite. This MDP monomer forms a nanolayer in the calcium salt-MDP structure due to an ionic bond with calcium from the dentin hydroxyapatite. In our study, we use the Clearfil Universal Bond system with self-etch mode, which includes an MDP monomer.

As a result of this study, it was shown that radiotherapy application affected dentin bond strength negatively. It was determined that the chitosan-containing agent and CHX application had a positive effect on the bond strength. Still, the chitosan-containing agent showed higher bond strength values than CHX, but this difference was not statistically significant.

This study has several limitations because it cannot fully simulate in vivo conditions. One of them is the use of the third molar. Also, this study is the first to evaluate this cavity disinfectant; cell culture studies may be required on the potential cytotoxicity of the gel.

Conclusions

Accepting the inherent limitations, it was concluded that the use of cavity disinfectant containing chitosan provides increased composite resin-dentin bond strength. However, the biological, physical, mechanical, and chemical radiation reactions in dental hard tissues should be investigated in detail, and the results should be supported by clinical studies.

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