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Investigation of the Effect of Bleaching on Discolored Teeth After Regenerative Treatment on Fracture Resistance of Teeth

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Abstract

Objective: This study aimed to evaluate the effect of bleaching discolored teeth after regenerative endodontic treatment on fracture resistance.

Methods: Forty human maxillary incisors were selected for the study and were divided into four study groups (n=10). Group 1 was called the positive control group, and no treatment was applied. In Group 2; root canals were enlarged to size 6 peezo reamer bur to mimic an immature tooth. The disinfection protocol was performed according to the regenerative endodontic treatment protocol of the American Association of Endodontists. 3 mm thick mineral trioxide aggregate (MTA) was placed at the cementoenamel margin and restored with a temporary restorative material. The samples in group 3 were restored with glass ionomer and composite resin. In Group 4 bleaching was applied with 35% hydrogen peroxide bleaching agent to the samples before restoration with glass ionomer and composite resin. Then each group was applied to the cervical fracture resistance test with a universal testing machine. The fracture resistance values were recorded in Newtons (N), and the obtained data were analyzed statistically.

Results: While the highest fracture strength was obtained in group 1 (1092 ±9.27N), the lowest fracture resistance was observed in group 2 (493.8 ±7.60N). The difference between all groups was statistically significant ($p<0.05$).

Conclusion: 35% hydrogen peroxide bleaching agent application after regenerative endodontic treatment reduces fracture resistance of the tooth.

Key Words: Fracture Resistance, Mineral Trioxide Aggregate, Regenerative Endodontics, Tooth Bleaching

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INTRODUCTION

Regenerative endodontic treatment is an exciting up-to-date treatment protocol of endodontics that provides revascularization that allows pulp-like tissue formation in both necrotic teeth with open apex and mature teeth with extensive endodontic lesions (1).

The foundations of regenerative treatment were first laid by Nygaard Ostyb with the demonstration of the formation of new vascularized tissue at the root tip of teeth, which have open apexes with periapical lesions (2). In this treatment protocol, which aims at regeneration, first of all, disinfection of the canal is provided, and then it is aimed to thicken the root dentin and close the apical opening by placing blood clots or progenitor cells that will initiate regeneration into the canal (3**)**. Despite all the positive aspects of the treatment, it is an undesirable result that the triple antibiotic paste (TAP) used in the disinfection phase causes discoloration on the crown (4**)**. It is known that the cause of the discoloration is the minocycline in the triple antibiotic paste (5). For this reason, in some studies, different antibiotic pastes that do not cause discoloration or treatment protocols that do not have a disinfection phase have been tried (6-8). However, it was reported that TAP is used in 51% of clinical cases (9).

On the other hand, mineral trioxide aggregate (MTA), also used as a coronal barrier in regenerative treatment, is also known to cause discoloration (3). This situation is often a concern for children or adolescent patients undergoing regenerative treatment and their families (10). Therefore, the American Association of Endodontists (AAE) recommends using low amounts of TAP and white MTA as a coronal barrier (11). Discoloration that occurs despite all precautions can be treated with intracoronal bleaching (12). Hydrogen peroxide (H_2O_2), often used in bleaching, is broken down into free radicals so that colored molecules break down into smaller colorless molecules. It is an agent with high oxidizing properties that provide bleaching (13). Unfortunately, in previous studies which examined the effects on the dentin surface, dentin fracture strength decreases, dentin stretching and harmful effects occur in shear forces, the calcium-phosphate ratio decreased, and cervical root it has been reported that the resorption (14-16). This situation is worrisome in terms of cervical resorption and fracture formation, especially in thin cervical dentin teeth with incomplete maturation (17). No study in the literature examined the effect of bleaching on teeth discolored due to regenerative endodontic treatment on cervical fracture formation. This in vitro study aimed to investigate the effect of bleaching on cervical fracture formation in teeth that underwent regenerative endodontic treatment protocols. The study's null hypothesis is that bleaching

will not affect cervical fracture resistance.

METHODS

The current research was confirmed by the ethics committee of Ordu University (2022/219). The sample size was calculated based on a power analysis using G*Power software version 3.1 (Universitat, Düsseldorf, Germany) at an alpha error probability of 0.05 and a power of 95% (effect size= 0.85). The power analysis showed that a totally 40 samples were required for groups. In the present study, 40 human mature maxillary incisors of similar dimensions extracted for periodontal or prosthetic reasons, without caries, previous canal treatment, restoration, and resorption were used. Non-probable sampling method was used in this study.

The periodontal tissues on the tooth surface were cleaned with the help of a periodontal curette. Periapical radiographs were taken from the teeth and evaluated for root canal calcification. Teeth showing calcification were excluded from the study. The teeth were evaluated for the presence of resorption fractures and cracks under a stereomicroscope (Leica SP1600; Leica, Wetzlar, Germany), and teeth with fractures, cracks, or resorption were excluded from the study. The root length of each tooth was standardized by cutting 10 mm from the apical to the cementoenamel margin.

Except for the positive control group (Group 1), the access cavity was opened for the remaining teeth. Preparation was made up to the number 70 k-file. Root canals were enlarged with from 1 to 6 number peezo reamer to mimic an incomplete maturation tooth (18). The canals were irrigated with 5ml of 1.5% sodium hypochlorite (NaOCl) during instrumentation. After the preparation was completed, the triple antibiotic paste, which equal portions of metronidazole (Eczacıbası, Istanbul, Turkey), ciprofloxacin (Biofarma, Istanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) mixed with distilled water (a powder/liquid ratio of 1:1:1/1) and with the lentulo spiral was placed in the canals. The access cavities were sealed with temporary restorative material (Cavit G; 3M ESPE, Seefeld, Germany), and the samples were held for three weeks in 100% humidity and 37 C^0 temperature. After three weeks, the TAP was removed from the root canals with conventional syringe irrigation using 17% Ethylene Diamine Tetra Acetic Acid (EDTA).

The samples were randomly divided into four groups (n=10):

***Group 1*:** This group of samples was selected as the positive control group and no treatment was applied.

Group 2: Samples were selected as the negative control group. 3 mm thick WhiteProRoot®MTA (Dentsply Sirona, York, PA, USA) was placed at the cementoenamel junction of the teeth with a pluger. Temporary restorative material was placed on it, waiting

for one day to set.

Group 3: The samples were restored with glass ionomer cement (3M ESPE, Seefeld, Germany) and composite resin (3M Filtek Z250; 3M ESPE, Seefeld, Germany) without bleaching after 3 mm thick WhiteProRoot®MTA was placed at the cementoenamel junction of the teeth with a pluger.

Group 4: After the glass ionomer cement was placed on the WhiteProRoot®MTA which was placed at the cementoenamel junction of the teeth, 35% H_2O_2 (Opalescence®Endo; Ultradent Products Inc., USA) was placed in the pulp chamber. The access cavity was covered with cotton and temporary restorative material and left for three days. After three days, the bleaching agent was replaced with fresh H_2O_2, and at the end of the second session, sufficient visible bleaching was achieved. The temporary restorative material was removed, and the teeth were restored with composite resin.

To mimic the periodontal ligament, the root surfaces were covered with wax before all teeth were embedded in acrylic resin blocks 2 cm wide and 4 cm in length. Before the acrylic polymerization was completed, the tooths were removed from the blocks, the wax was cleaned, the root surfaces were covered with polyvinyl siloxane impression material (Elite HD; Zhermack, Italy), and the roots of the teeth were again embedded in the blocks. A distance of 2 mm was left between the cementoenamel junction and the acrylic block to simulate the relationship between the alveolar bone and tooth. The samples were placed on the tip of the universal testing machine (Autograph AGS X; Shimadzu Co, Japan.) with a diameter of 3 mm in such a way that the crowns were at an angle of 135^0 degrees to perform the fracture test (Fig. 1). To mimic crown trauma, a force of 1 mm/min was applied on the palatine of the teeth, 3 mm over the cementoenamel junction. When the teeth were broken, the force was recorded in newtons (N).

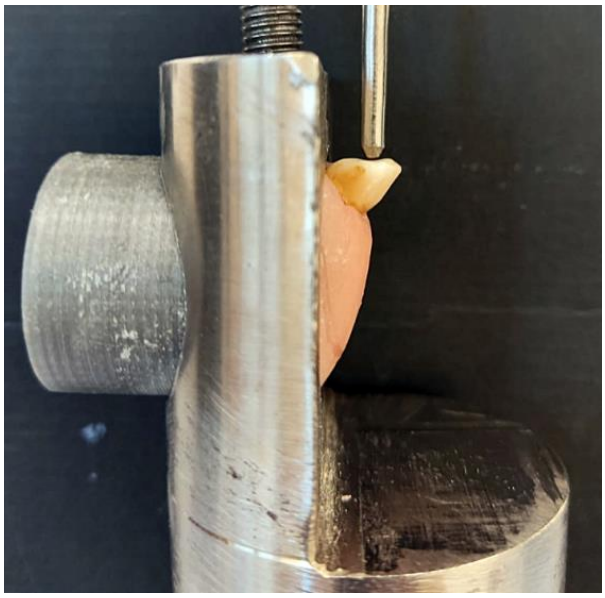

Figure 1: The samples were placed on the tip of the universal testing machine (Autograph AGS X; Shimadzu Co, Japan.)

The variables of the study are permanent restoration and bleaching application. The primary measurement of the study is the fracture resistance of teeth. According to the Shapiro-Wilk normality test, the groups'

fracture resistance values were normally distributed ($p > 0.05$). The fracture resistance results were analyzed by one-way analysis of variance. Finally, the mean values were compared using Tamhane's multiple comparison tests ($\alpha = 0.05$). All computational work was performed using SPSS statistical software (SPSS v18.0; SPSS Inc. Chicago, IL).

RESULTS

Fractures occurred in horizontal and vertical directions in all teeth. The results of the fracture test are shown in Table 1. The highest fracture resistance value was obtained in group 1 (1092 ±9.27 N), while the lowest fracture resistance value was obtained in group 2 (493.8 ±7.60 N). The fracture resistance value in group 4 with bleaching group (688.8 ±4.70 N) was lower than group 3 (887.8 ±4.46 N) without bleaching. The difference between all groups was statistically significant ($p<0.05$) (Table 1).

Table1. The mean and standard deviation (SD) of fracture resistance values (N) of test groups

GROUPS	N	Mean ± SD
Group 1	10	1092 ±9.27 [a]
Group 2	10	493.8 ±7.60 [d]
Group 3	10	887.8 ±4.46 [b]
Group 4	10	688.8 ±4.70 [c]

*Different superscripts mean statistically significant difference. Significant at $p<0.05$

DISCUSSION

Regenerative endodontic treatment protocol allows the apexification of a tooth with an infected pulp whose apex is not closed without compromising its fracture resistance (19). Although there was no standard procedure for this treatment, disinfection with antibiotic pastes is the most critical phase (20). Despite the disadvantage of discoloration, the main reason why triple antibiotic paste is widely used is that it can penetrate 350 µm into the dentinal tubules and eliminate microorganisms there (21). Although many studies showed that regenerative endodontic treatment causes discoloration, no study examined the effects of bleaching on tooth structure after regenerative endodontic treatment (3,5,12). Previous studies have shown that using hydrogen peroxide with the intracoronal bleaching method is used to bleach teeth discolored due to regenerative endodontic treatment (22-24). Previous studies which evaluated the effect of intracoronal bleaching with H_2O_2 on dentin surface reported that dentin break of strength decreased, dentin stretching and shearing in their strength damaging effects formed, the calcium-phosphate rate decreased, and cervical root resorption (14-16,25). This situation is thought to pose a fracture risk for immature teeth with thin dentin walls. In the present study, we aimed to evaluate the fracture resistance of intracoronal bleached teeth with H_2O_2 after discoloration due to regenerative treatment with artificially simulated immature roots. According to the results of the present study, the fracture resistance value of the intracoronal bleaching group was obtained statistically lower than the non-bleaching

group. Therefore, the null hypothesis of the present study was rejected. A tooth prepared for endodontic treatment is considered to be more fragile than a vital tooth. This may be due to access cavity preparation, root canal enlargement, and post-cavity preparation (24). In the present study, root fracture resistance was found to be the lowest in the group with root canal enlargement and no restoration (26).

Lado et al. stated that bleaching agents penetrate dentin and cause cervical resorption by denaturation between enamel and cementum (27). The present study's lower fracture resistance in the intracoronal bleaching group parallels this study. In previous studies, it has been shown that H_2O_2 reduces the microhardness of dentin by degrading the matrix metalloproteinase enzyme, but this has no clinical significance (28,29). These results are inconsistent with our study. This may be due to the higher concentration of hydrogen peroxide used in the present study. To avoid this adverse effect, low-concentration hydrogen peroxide might be recommended (29). However, using hydrogen peroxide in low concentration causes the tooth to be exposed to a bleaching agent for a longer period due to more treatment sessions. Therefore, in this study, a high concentration of hydrogen peroxide was used for a short time.

It has been reported that the cause of discoloration in regenerative treatments with alternative pastes instead of antibiotic pastes containing minocycline may be due to the use of MTA as a coronal barrier (30-32). To eliminate this situation, white MTA was used in the present study. However, in the clinical setting, blood in contact with MTA can also cause coronal discoloration (5). The present study under in vitro conditions suggests that the cause of the discoloration is due to the minocycline in the antibiotic paste. Although some previous studies reported that intracoronal bleaching of teeth discolored due to regenerative treatment was not satisfactory in clinical situations (33,34). Küçükekenci et al. reported that bleaching was successful in their in vitro study (24).

Samples must be placed in specially prepared moulds for fracture tests. For this purpose, auto-polymerizing acrylic or polymethyl methacrylate can be used (35). Since the forces coming to the teeth in the oral environment are buffered with periodontal tissues, in the experimental setup, the roots were covered with polyvinylsiloxane impression material to mimic the periodontal ligament and embedded in auto-polymerizing blocks that mimic bone (36). In the fracture test, a 135^0 loading angle was chosen by the relationship between the maxillary and mandibular incisors (18). In fracture resistance studies, human teeth are generally used, but the parameters should be minimized as much as possible for standardization. Therefore,

buccolingual and mesiodistal widths close to each other and standard-sized human incisors were used in this study (37).

CONCLUSIONS

This study, which shows the negative effect of bleaching of discolored teeth due to regenerative endodontic treatment on cervical fracture resistance, recommends the application of treatment protocols that preserve the original color instead of bleaching the discoloration.

Ethics Committee Approval: Ethics committee approval was received for this study from Ordu University Clinical Research Ethics Committee (2022/219)
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