Adhesive Resistance of Bovine Teeth with Dental Bleaching with Ozone and Hydrogen Peroxide with/without Calcium *

Resistencia adhesiva de dientes bovinos con aclaramiento dental con ozono y peróxido de hidrógeno con/sin calcio

Resistência adesiva de dentes bovinos com clareamento dental com ozônio e peróxido de hidrogênio com/sem cálcio

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ABSTRACT

Background: Tooth whitening is a widely used aesthetic technique; however, some bleaching agents can affect enamel adhesive strength. There is interest in determining the effects of ozone and hydrogen peroxide, with and without calcium, on this property. **Purpose**: To evaluate the differences in enamel adhesive strength in bovine teeth subjected to tooth whitening with ozone, hydrogen peroxide with calcium, and hydrogen peroxide without calcium. **Methods**: An experimental study with an *in vitro* design was used. 75 bovine lower anterior incisors were divided into three groups (n=25 per group). The respective bleaching treatments were applied, and adhesive strength was measured using a digital universal testing machine at a speed of 0.5 mm/min. Measurements were taken at 24 hours, 7, 14, and 21 days after treatment. Data were analyzed using ANOVA and Tukey's *post hoc* test. **Results**: Statistically significant differences were observed between groups at 24 hours ($p = 0.0044$) and 7 days ($p = 0.0026$). The highest adhesive strength was recorded with 35% hydrogen peroxide with calcium at 21 days (11.50 ± 2.27 MPa). In all three groups, strength progressively increased over time. **Conclusion**: The type of bleaching agent and the time elapsed after treatment influence the adhesive strength of the enamel. Hydrogen peroxide with calcium showed the best performance at 21 days.
Keywords: adhesive strength; biophysics; bleaching agents; dental adhesives; dental materials; dentistry; hydrogen peroxide; ozone; tooth whitening

RESUMEN

Antecedentes: El blanqueamiento dental es una técnica estética ampliamente utilizada; sin embargo, algunos agentes blanqueadores pueden afectar la resistencia adhesiva del esmalte. Existe interés por determinar los efectos del ozono y del peróxido de hidrógeno, con y sin calcio, sobre dicha propiedad. **Objetivo**: Evaluar las diferencias en la resistencia adhesiva del esmalte en dientes bovinos sometidos a aclaramiento dental con ozono, peróxido de hidrógeno con calcio y peróxido de hidrógeno sin calcio. **Métodos**: Estudio experimental con diseño *in vitro*. Se utilizaron 75 dientes incisivos anteroinferiores de bovino distribuidos en tres grupos (n=25 por grupo). Se aplicaron los tratamientos blanqueadores respectivos y se midió la resistencia adhesiva mediante una máquina de ensayo universal digital a una velocidad de 0,5 mm/min. Las mediciones se

realizaron a las 24 horas, 7, 14 y 21 días posteriores al tratamiento. Los datos se analizaron con ANOVA y la prueba *post hoc* de Tukey. **Resultados**: Se observaron diferencias estadísticamente significativas entre los grupos a las 24 horas (p = 0,0044) y a los 7 días (p = 0,0026). La mayor resistencia adhesiva se registró con el peróxido de hidrógeno al 35 % con calcio a los 21 días (11,50 ± 2,27 MPa). En los tres grupos, la resistencia aumentó progresivamente en el tiempo. **Conclusión**: El tipo de agente blanqueador y el tiempo transcurrido tras el tratamiento influyen en la resistencia adhesiva del esmalte. El peróxido de hidrógeno con calcio mostró el mejor desempeño a los 21 días.
Palabras clave: adhesivos dentales; agentes blanqueadores; biofísica; blanqueamiento dental; materiales dentales; odontología; ozono; peróxido de hidrógeno; resistencia adhesiva

RESUMO

Antecedentes: O clareamento dentário é uma técnica estética amplamente utilizada; Entretanto, alguns agentes clareadores podem afetar a força adesiva do esmalte. Há interesse em determinar os efeitos do ozônio e do peróxido de hidrogênio, com e sem cálcio, nessa propriedade. **Objetivo**: Avaliar as diferenças na resistência adesiva do esmalte de dentes bovinos submetidos ao clareamento dental com ozônio, peróxido de hidrogênio com cálcio e peróxido de hidrogênio sem cálcio. **Métodos**: Estudo experimental com delineamento *in vitro*. Foram utilizados 75 dentes incisivos inferiores anteriores bovinos, distribuídos em três grupos (n=25 por grupo). Os respectivos tratamentos de clareamento foram aplicados e a resistência adesiva foi medida usando uma máquina de teste universal digital a uma velocidade de 0,5 mm/min. As medições foram feitas 24 horas, 7, 14 e 21 dias após o tratamento. Os dados foram analisados usando ANOVA e teste post hoc de Tukey. **Resultados**: Foram observadas diferenças estatisticamente significativas entre os grupos em 24 horas (p = 0,0044) e em 7 dias (p = 0,0026). A maior resistência adesiva foi registrada com 35% de peróxido de hidrogênio com cálcio em 21 dias (11,50 ± 2,27 MPa). Em todos os três grupos, a resistência aumentou progressivamente ao longo do tempo. **Conclusão**: O tipo de agente clareador e o tempo decorrido após o tratamento influenciam na resistência adesiva do esmalte. O peróxido de hidrogênio e cálcio apresentou o melhor desempenho em 21 dias.
Palavras-chave: adesivos dentários; agentes de branqueamento; biofísica; clareamento dental; força adesiva; materiais odontológicos; odontologia; ozônio; peróxido de hidrogênio

INTRODUCTION

The quest for whiter teeth is a common concern for many patients visiting the dental office. This is because even a slight change in the color of one or more teeth can negatively affect their aesthetic expectations and perception of their own dental appearance (1). Dentistry offers various corrective techniques and treatments to address this issue, including resin restorations, dental veneers made of porcelain or other materials, and crowns made using various techniques. Teeth whitening, also known as bleaching, is an increasingly popular method and a more conservative and manageable option to resolve this difficulty. This procedure is commonly performed using hydrogen peroxide gels in different concentrations (2). Bleaching is a cosmetic treatment used to lighten the tone of teeth and enhance the aesthetics of the smile.

Hydrogen peroxide is an unstable, low-molecular-weight free radical that penetrates enamel and dentin by diffusion. Organic pigment molecules in tissues are broken down and converted into simple hydrophilic molecules through an oxidation-reduction process. When these molecules come into contact with water, they are eliminated from the dental tissue, producing the desired whitening effect (3). Color problems affect the appearance of teeth. Dental stains generally result from the accumulation of chromogenic substances on the external surface of the tooth. These substances must be carefully evaluated to predict the whitening outcome, as some stains respond better to the process than others (4). The use of hydrogen peroxide largely releases H+ ions, which cause tooth sensitivity due to the decrease in pH and acidification of the teeth (5).

This improves tooth tone. Although there is little evidence of ozone's effectiveness as a whitening agent, its use is attractive because it does not cause tooth sensitivity, unlike peroxides. Ozone is an unstable gas that dissociates into oxygen, does not release free hydrogen, and prevents acidification of tooth enamel. This eliminates the possibility of tooth sensitivity. Therefore, ozone is an attractive option for patients with high sensitivity or a low pain threshold (6).

Research has identified several problems associated with tooth whitening techniques. These include soft tissue damage, changes in tooth structure, burns, tooth hypersensitivity, and a significant decrease in adhesion levels. After tooth whitening treatment, whether with peroxide gels or ozone gas, the presence of oxygen free radicals in tooth structures makes tooth adhesion difficult (6).

To determine adhesive efficacy, the micro-tensile test is used. This method measures the adhesion strength in small areas of 0.5 mm^2 to 1 mm^2 and allows for an accurate assessment of the bond strength between tooth structure and restorative material. The test detects adhesive failures and provides a realistic analysis of adhesion. Bovine teeth constitute the gold standard due to their macroscopic and microscopic structural similarity to human teeth (7). In this context, the objective of the present study was to evaluate the adhesive strength to tooth enamel after bleaching with ozone and hydrogen peroxide, with and without calcium, in bovine teeth, with the aim of improving post-bleaching dental treatments.

MATERIALS AND METHODS

An experimental study with an *in vitro* design was conducted. The sample included a total of 75 bovine lower anterior incisors extracted within the previous three months, which were assigned to three groups: 25 teeth treated with ozone, 25 teeth treated with calcium hydrogen peroxide, and 25 teeth treated with calcium hydrogen peroxide. Only lower anterior incisors without dental caries, enamel hypoplasia, or crown fractures were considered.

Sample size calculation:

$$n = \frac{(Z_\alpha + Z_\beta)^2 * (S)^2}{(d)^2}$$

In which:

n = number of teeth needed in each sample group
$Z\alpha = 1.96$
$Z\beta = 0.8$
S = 4.6
d = 1.7

The calculated sample size was 25 bovine teeth per group.

To obtain the sample teeth, authorization was obtained from the Dental Clinic of the Federico Villarreal National University in Lima, Peru, according to the established schedule. Training for the research staff took place between March and May 2022. In addition, a High Technology Laboratory Certificate (ISO/IEC Standard: 17025) was obtained.

Sample Preparation

Freshly extracted incisors were measured and stored in distilled water refrigerated at 4°C, with replacement every 4 days. A cervical cross-section was then made with a Disco Flex Diamantado Dupla Face - 7020 diamond blade (KG-Sorensen, Barueri, Brazil) (Figures 1 and 2).

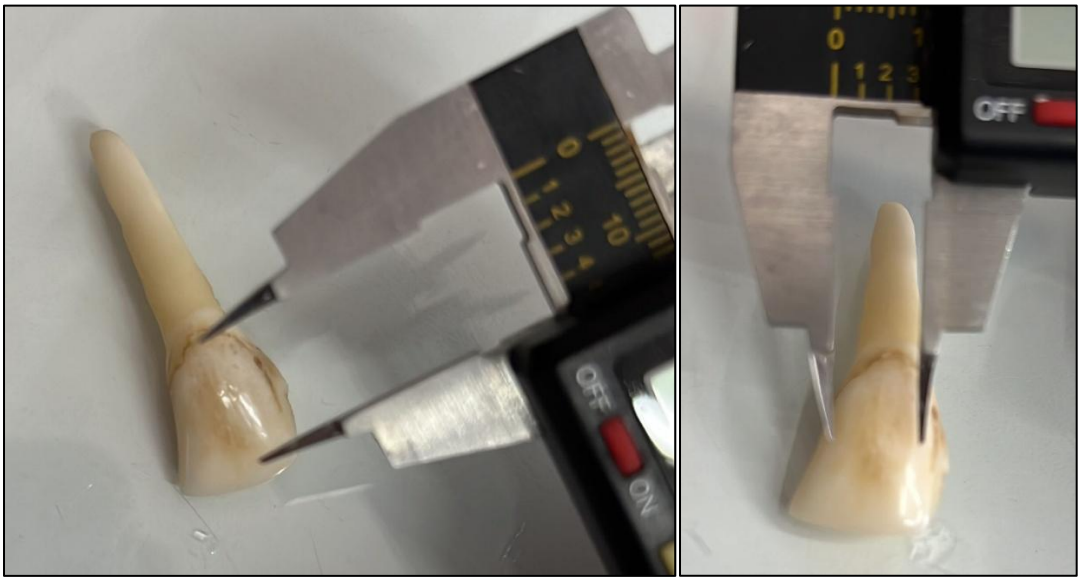

FIGURE 1
Measurement of the Lower Incisors
Source: the authors.

FIGURE 2
Lower Incisors Immersed in Distilled Water
Source: the authors.

The pulp chamber was rinsed with distilled water and then filled with glass ionomer (Fuji II® Universal Restorative Glass Ionomer, GC, Tokyo, Japan) until it was completely covered. The teeth were positioned on the model (Dental Simulator, Foshan Huiyigang Medical Equipment Co., Ltd., Guangdong, China) simulating a maxillary arch. The center of the buccal surface of the teeth was then smoothed to obtain a flat area using 1/2" coarse Sof-Lex polishing discs -1982C- (3M ESPE®, St. Paul, MN, USA). Before the application of the bleaching agent, all teeth in their respective arches received dental prophylaxis with pumice stone and low-speed brushes.

Dental Whitening

Group A

35% hydrogen peroxide Whiteness HP Maxx® (FGM, Joinville, Brazil) was used, following the manufacturer's protocol. The teeth were prophylactically treated, and a desensitizer (KF 2% potassium nitrate with 2% sodium fluoride) was applied to the enamel for 10 minutes; the teeth were then washed and dried. The whitener was applied in a 3:1 ratio, mixing 18 drops of phase 1 hydrogen peroxide with 6 drops of phase 2 thickener for all 20 teeth. The gel was applied in the thinnest possible layers, stirring with a mini-brush to avoid bubbles, and left on for 15 minutes before removal. The gel was then washed and polished with FGM® Diamond felt discs and FGM® Diamond Excel polishing paste.

Group B

35% hydrogen peroxide with Whiteness HP Blue® (FGM, Joinville, Brazil) desensitizer (calcium) was used, following the manufacturer's instructions. First, the KF® desensitizer with 2% potassium nitrate and 2% sodium fluoride was applied for 10 minutes. The gel was then washed and dried. To mix the whitening gel, both phases were transferred through interconnected syringes, repeating this process four times per side (eight times total) until a homogeneous mixture was obtained. A 1.2 g syringe was sufficient for 20 teeth, applied in thin layers. The gel remained on the teeth for 40 minutes, and bubbles were removed when present. Finally, the gel was washed and polished using FGM® Diamond felt discs and FGM® Diamond Excel polishing paste.

Group C

In the ozone (O_3)-treated group, a high-precision digital splint was 3D-printed over the model with the bovine teeth positioned, leaving a 5 mm vestibular space. This allowed the gas to pass through the front of the teeth via feeder hoses. An ozone generator (0.5 ppm), supplied with medicinal oxygen at 1.5 to 2 liters per minute, was connected via two inlets located on the front of the splint. On the back, two outlets connected to the dental equipment's saliva ejector allowed the ozone to pass through under negative pressure, thus closing the gas circuit. This procedure lasted 30 minutes. After applications with 35% hydrogen peroxide, 35% hydrogen peroxide with desensitizer, and ozone, the specimens were washed with copious amounts of distilled water to remove bleach residues, and subsequently stored again in saliva, which was renewed every three days at 37°C.

Adhesion Process

The specimens were mounted on small 15 mm x 15 mm acrylic blocks. The enamel was cleaned with pumice paste. Subsequently, 37% phosphoric acid (Total Etch – Scotchbond®, Merchant to enamel) was applied for 15 seconds and washed with water for 10 seconds. A thin layer of 3M® Adper® Single Bond Plus adhesive (3M ESPE, St. Paul, MN, USA) was then applied with a microbrush (Young Innovations, Inc.®, IL, USA), followed by gentle air pressure for 3 seconds, and light-cured for 10 seconds with a third-generation LED lamp (Valo®, Ultradent, USA). Finally, Filtek Bulk Fill® resin blocks (3M ESPE®, St. Paul, MN, USA) were placed in a single layer, using an 8 mm long x 8 mm wide x 4 mm thick matrix, which was photopolymerized with the same LED lamp at an intensity of 1200 mW/cm² for 20 seconds.

Handling of Specimens

The enamel-bonded resins were cut using an Isomed® machine (Buehler Ltd., Lake Bluff, IL, USA). Horizontal and vertical cuts were made with a water-cooled, low-speed diamond disc, changing the disc

every five cuts. The microbar dimensions were 1 mm × 1 mm × 8 mm, which were verified with a digital vernier caliper (Mitutoyo®, Japan).

Adhesion Resistance Test

Once the samples were obtained, they were stored in distilled water for 24 hours. Subgroups were selected from each group (A, B, and C) and subjected to adhesion strength testing at 24 hours, 7 days, 14 days, and 21 days. These tests were performed using a digital universal testing machine (CMT-5L, series 7419, LG, Seoul, Korea), equipped with SmartTest® software, at a speed of 0.5 mm/min. The bond strength values obtained were analyzed in megapascals (MPa), calculated as force (kg) divided by area (cm^2) (Figure 3).

FIGURE 3
Micro-tension Machine
Source: the authors.

The data obtained were recorded in a Microsoft Excel 2019® spreadsheet and subsequently analyzed using SPSS® (Statistical Package for the Social Sciences Inc., IBM, NY, USA), version 24.0. Measures of central tendency and dispersion were used for descriptive analysis. ANOVA was used for comparative analysis, followed by Tukey's *post hoc* test to assess differences between groups.

RESULTS

In the group treated with 35% hydrogen peroxide without calcium, the lowest adhesive strength was observed at 24 hours (5.58 ± 1.32), while the highest was recorded at 21 days (11.29 ± 3.60). For the group with 35% hydrogen peroxide with calcium, the lowest measurement was also evident at 24 hours (7.32 ± 2.09) and the highest at 21 days (11.50 ± 2.27). Regarding the group treated with ozone, the lowest resistance was obtained at 24 hours (6.80 ± 2.00), while the highest was observed at 21 days (10.23 ± 2.83) (Table 1).

TABLE 1
Adhesive Resistance according to Whitening Agents

Whitening Method	24 hours		7 days		14 days		21 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
35% hydrogen peroxide without calcium	5.58	1.32	6.88	1.87	10.32	3.55	11.29	3.6
35% hydrogen peroxide with calcium	7.32	2.09	8.68	2.26	9.21	2.18	11.5	2.27
Ozone	6.8	2	8.39	1.45	9.03	2.29	10.23	2.83

Source: the authors.

Likewise, there were statistically significant differences between groups at 24 hours ($p = 0.0044$), particularly between 35% hydrogen peroxide without calcium and 35% hydrogen peroxide with calcium ($p = 0.004$). Also at 7 days, there were significant differences ($p = 0.0026$), both between the peroxide group without calcium and the group with calcium ($p = 0.004$), and between the group without calcium and the ozone-treated group ($p = 0.019$). In contrast, no significant differences were observed between groups at 14 days ($p = 0.2061$) and 21 days ($p = 0.2744$) (Table 2).

TABLE 2
Adhesive Resistance According to Bleaching Agent Time

Whitening Method	24 hours		7 days		14 days		21 days	
	Test	p	Test	p	Test	p	Test	p
35% hydrogen peroxide without calcium								
35% hydrogen peroxide with calcium	F = 5.87	0.0044	F = 6.46	0.0026	F = 1.61	0.2061	F = 1.32	0.2744
Ozone								
Post-hoc: Bonferroni test								
HP/HP+C*	1.7348	0.004	1.7916	0.004	-	-	-	-
HP/Ozone	-	-	1.5104	0.019	-	-	-	-
HP+C/Ozone	-	-	-	-	-	-	-	-

Source: the authors. * HP: hydrogen peroxide without calcium. HP+C: hydrogen peroxide with calcium.

Statistically significant differences in adhesive strength were observed over time in all three bleaching agent groups ($p < 0.001$). In the calcium-free hydrogen peroxide group, differences were detected at almost all evaluation intervals. In the calcium group, the most notable differences were found between 24 hours and 21 days. In the ozone-treated group, significant differences were observed primarily between 24 hours and subsequent days. These findings demonstrate that adhesive strength tends to increase over time, regardless of the agent used (Table 3).

TABLE 3
Adhesive Strength According to Bleaching Agent Over Time

Time	35% hydrogen peroxide without calcium		35% hydrogen peroxide with calcium		Ozone	
	Test	**p**	**Test**	**p**	**Test**	**p**
24 hours 7 days 14 days 21 days	F = 21.70	0,000	F = 14.97	0,000	F = 11.70	0,000
***Post-hoc*: Bonferroni test**						
24 hours / 7 days	1.302	0.036	-	-	1.594	0.018
24 hours / 14 days	4.739	0,000	-	-	2.232	0.002
24 hours / 21 days	5.706	0,000	4.179	0,000	3.432	0,000
7 days / 14 days	3.437	0.002	-	-	-	-
7 days / 21 days	4.404	0,000	2.82	0.002	-	-
14 days / 21 days	-	-	2.284	0.008	-	-

Source: the authors.

DISCUSSION

Adhesive strength on the surface of tooth enamel after whitening is one of the main problems, due to the chemical changes that occur after the application of ozone gas, hydrogen peroxide with or without calcium. These changes affect the durability of dental treatments. Adhesive strength was found to vary over time between the groups treated with ozone, 35% hydrogen peroxide without calcium, and with calcium. The differences were statistically significant at 24 hours ($p = 0.0044$) and 7 days ($p = 0.0026$), while no significant differences were observed at 14 and 21 days ($p = 0.2061$ and $p = 0.2744$, respectively).

The highest mean was obtained with 35% hydrogen peroxide with calcium at 21 days (11.50 MPa). This result does not coincide with that reported by Santos *et al*. (2019) (8), who recorded a mean of 9.22 MPa at 28 days for the same agent. Likewise, the findings do not match the study by Marski *et al*. (2022) (9), who evaluated the adhesive strength to tooth enamel using 35% hydrogen peroxide with and without calcium. When performing restorative procedures in this study at 24 hours and 7 days after bleaching, they observed no significant differences between the groups, as both showed similar strength values.

There were differences between the bleaching agent groups at 24 hours and 7 days. This differs from that reported by Yanık *et al*. (10), who showed that ozone had no negative effect on bond strength and found no statistically significant differences between the different time intervals. Similar results were reported by Çelik *et al*. (11), who concluded that ozone bleaching did not affect adhesive strength. Similarly, Dinc and Mujdeci (12) found that the bond strength of the control group was comparable to that of the group treated with ozone for 20 seconds.

A study conducted by Alaghehmand *et al*. (13) showed no differences in adhesion strength between enamel subgroups subjected to hydrogen peroxide bleaching and a non-bleached group. Adhesive failures were observed in all groups. Furthermore, it was shown that the application of ozone did not alter the enamel morphology.

The results of the present investigation coincide with those reported by Cheng *et al*. (14). These researchers found that, compared with a control group without whitening treatment, a group treated with 40% hydrogen peroxide presented a higher percentage of adhesive failures.

Statistically significant differences were found between the bleaching agent groups at 24 hours, 7 days, 14 days, and 21 days. This finding is consistent with the results of Baia *et al*. (15), who reported that all

experimental groups showed statistical differences except at 14 days, when the teeth were stored in artificial saliva for 7 days. No significant differences were found at 21 and 28 days, regardless of storage time. The study concluded that prolonged bleaching with hydrogen peroxide decreases adhesive strength, regardless of the time of application.

The results are consistent with those reported by Santos *et al*. (8), who found no differences in adhesive strength between the 24-hour and 7-day storage periods after bleaching with calcium hydrogen peroxide at 14 days. However, at 28 days, differences were evident between these periods. This suggests that prolonged bleaching reduces adhesive strength, regardless of storage time in artificial saliva.

CONCLUSIONS

The results of this study suggest that the adhesive strength of bovine tooth enamel varies depending on the bleaching agent used and the time elapsed after treatment. 35% hydrogen peroxide with calcium showed the highest adhesive strength at 21 days (11.50 ± 2.27 MPa), being significantly superior to hydrogen peroxide without calcium. Regarding time, the best overall results were observed at 21 days ($p = 0.2744$). Statistically significant differences in adhesive strength were found between 24 hours and 21 days for both the group treated with calcium-free hydrogen peroxide ($p = 0.000$) and the group treated with ozone ($p = 0.000$). These findings suggest that the time after treatment and the type of bleaching agent significantly influence enamel adhesive strength.

RECOMMENDATIONS

Further research on the adhesive strength of enamel in teeth treated with various bleaching agents, such as ozone and calcium-containing and calcium-free hydrogen peroxide, is recommended in order to optimize the adhesion of restorative materials. Comparative histological studies analyzing structural changes in enamel based on the agent used are also relevant. Research is also needed to identify potential factors influencing changes in enamel adhesion.

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