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FRACTURE RESISTANCE OF ENDODONTICALLY TREATED TEETH BLEACHED WITH EXTRACT OF SPIRULINA ALGAE (AN- INVITRO STUDY)

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ABSTRACT

Background: Endodontically treated teeth are subjected to discoloration which must be treated for aesthetic purposes without weakening of tooth structure.

Methods: Intra-coronal bleaching using different bleaching agents was tested for affecting the fracture resistance of teeth in three different groups, group 1(n=17) treated with sodium perborate, group 2 (n=17) treated with spirulina extract using photodynamic theory, and group 3 (n=17) control group without bleaching. Fracture resistance of single-rooted extracted premolar teeth was tested using a universal testing machine under a compressive load of 135 degrees on the palatal aspect of the tooth until fracture.

Results: Statistical analysis revealed no significant difference between bleaching protocols on fracture resistance of endodontically treated teeth, but they were significantly lower than the nonbleached control group.

Conclusions: Bleaching of endodontically treated may compromise the fracture toughness and structural integrity of dentine after root canal treatment with both the conventional bleaching protocol and the photodynamic theory of bleaching.

KEYWORDS: Spirulina algae, Bleaching, Fracture resistance, Photodynamic theory.

INTRODUCTION

Intracoronal bleaching (walking bleach) is a conservative esthetic treatment of discolored endodontically-treated teeth. The most prevalent bleaching materials promote an oxidation-reduction reaction as in the hydrogen peroxide (HP) reaction with the teeth, in several concentrations or activation methods.[(1)]

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Different oxidizing agents such as Hydrogen peroxide (HP) which is availabe in two different concentrations ranging between 30% and 35% have the ability to penetrate dentinal tubules and release oxygen that in turn reacts with the organic and inorganic double bonds and break them to remove stains. [2]

Sodium perborate (SP) or carbamide peroxide (CP) are another chemicals that are used also as bleaching agents with HP as a final product and both have been used for intra-coronal bleaching.[3, 4]

Light sources are now used to activate peroxides by generation of photons of light energy that in turn activate the photosensitizer to generate reactive species, these sources are in the form of light emitting diode, plasma arc lamp, halogen lamp and lasers. [5]

Lasers have gradually gained much recognition in teeth bleaching as it acts as a source of heat and light increasing the breakdown of reactive oxygen species (ROS) and accelerating the process. [6]

The adverse effects of a bleaching agent are credited mainly to high concentrations of HP that cause enamel and dentin demineralization with subsequent tooth microhardness reduction.[7]

These changes may negatively affect the fracture resistance of bleached teeth, especially if the tooth was endodontically treated as it is predicted to be of lower fracture resistance and hardness than vital teeth. [8, 9]

Nowadays, it is recommended to use bleaching protocols with a lower concentration of peroxide in comparison to traditional bleaching materials, in order not to negatively affect the dentine structure and properties.[10] Photodynamic therapy (PDT) is a non-invasive therapeutic modality, that is used in the treatment of many clinical indications. PDT is now suggested to activate different materials including bleaching agents. [11, 12]

The idea of using photodynamic therapy for teeth bleaching was introduced to the dental field by adding a photosensitizer to peroxide bleaching agents, such as rhodamine B, β-carotene, and titanium dioxide photocatalyst. PDT uses a light source of low intensity to produce ROS through the oxidation process. [8]

An ideal photosensitizer ought to demonstrate a minimal toxic effect on host cells, produce ROS immediately, and should have great solubility in water with an excellent shelf life in order not to lose its efficacy by storage. [13]

Toluidine blue (TB) and C-Phycocyanin (C-PC) are photosensitizers that closely fulfil these requirements using them in dentistry is still rare, and still questionable.[14, 15]

This in vitro study was conducted to compare the fracture resistance of endodontically-treated teeth intra-coronally bleached using a novel photosensitizer (C-PC) with PDT protocol to a traditional bleaching protocol using sodium perborate.

Null hypothesis: the fracture resistance of the endodontically treated teeth bleached using the PDT will not be affected in comparison to the traditional methods of bleaching.

MATERIAL AND METHODS

Materials

1. Sodium Perborate (SP) (Morgan Speciality Chemicals)
2. C-Phycocyanin (C-PC) (National Research Centre)

Methods:

Samples selection

Single-rooted extracted permanent human teeth (n=51) with mature root apices were used in this in vitro study. All teeth were mechanically scaled to remove any remaining bone, calculus, or periodontal ligament using an ultrasonic scaler. Each tooth was placed in NaOCl (5.25%) for two hours for surface cleansing and then deposited in distilled water until use.(16)

Endodontic treatment

Preoperative radiographs were taken to ensure root canal patency. The teeth access was opened using a #12 diamond bur under high-speed water spray cooling and the working length (WL) was established by subtracting 1 mm from the distance between the reference point and the tip of the k-file #15 that just protruded from the apical foramen. Preparation of the canal was done using the crown-down technique with the rotary M-Pro nickel-titanium instruments (IMD Company) following the manufacturer's instructions up to #35 instrument.

The M-Pro system was connected to an endodontic micro-motor (Wismy). Irrigation was done for each canal with 2 ml of 5.25% sodium hypochlorite (NaOCl) at each file size using a 27-gauge needle. After preparation, irrigation with 5 ml of ethylenediaminetetraacetic acid (EDTA) in concentration of 17% for 60 seconds was performed for debris removal.(17)

Afterwards, obturation with gutta-percha and root canal sealer (MetaBiomed resin sealer) was done using lateral condensation technique. A heated plugger was then used to remove two millimetres of the filling at the area below the cementoenamel junction and then covered with 1 mm thick resin-modified glass-ionomer cement (GC-FUGI, Tukyo, Japan) for cervical seal. In the remaining area of pulp chamber a cotton pellet was inserted and the cavity was then filled with temporary restoration (Orafil-G).(18)

Mounting of the endodontically treated teeth

Teeth were then implanted in acrylic resin (acrostone, Egypt) to the level of cementoenamel junction with the use of a plastic matrix (16.5 mm in width × 20.0 mm in length). They were kept intact for 24 hours to allow resin polymerization. Afterwards, temporary restoration was removed from the pulp chamber and was then irrigated with 5.0 ml of 2.5% NaOCl. Phosphoric acid (37%) was then applied for 15 seconds and then rinsed with distilled water for 60 seconds to remove smear layer. (19)

Bleaching materials

PHYCOCYANIN extract (C-PC)

Algae were milled in 100 mL of 0.1M phosphate buffer solution (pH: 6.8) (Sigelman and Kycia 1978), and 10 mL of Tris-HCl in the presence of acid-washed neutral sand and then filtered. The mixture was further subjected to freezing (-20 °C for 30–100 minutes) and thawing for 3–10 freeze-thaw cycles in total, stirred at 150 rpm at 4°C for 30 minutes, sonicated ten times. C-phycocyanin absorbance ratio were calculated using the spectrophotometry-based methods.

Phycocyanin concentration in mg/mL was estimated from the optical densities at 652 and 620nm, using the following Equation:

$$C\text{–}PC\left(\frac{mg}{ml}\right)=\frac{OD\ 620\text{-}\ 00474OD652}{5.34}$$

This ratio determined the purity of C-phycocyanin preparations. Purity ratio of C-Phycocyanin = A620 / A280m 0°C with ten seconds of sonication, ten-second intervals, centrifuged at 4°C for 8 minutes at 7000 rpm, and the blue-colored supernatant was taken for further investigations.(20)

SODIUM PERBORATE (SP)

Sodium perborate bleaching material was prepared by mixing in a ratio of 2 g of powder to 1 mL of distilled water.

Intra-coronal bleaching of endodontically treated teeth.

The teeth (n=51) were allocated into 3 groups according to the bleaching materials used:

Group I: Pulp chambers (n=17) were treated with sodium perborate

Group II: Pulp chambers (n=17) were treated with photosensitizer (5% C-Phycocyanin). C-Phycocyanin was activated for 3 min in continuous mode using a 625-nm diode laser (Lasotranix Company) 220 mW output and power density of 0.34 w/cm2. (21)

Group III (Control group): The teeth were not bleached (n=17).

The access cavities were then sealed with temporary cement. All the procedure were then repeated after 7 and 14 days. samples were saved in artificial saliva at 37°C that was changed regularly until the fracture resistance tests were conducted.

(6) Fracture resistance test:

All the teeth were placed on a precisely designed inclined position device (45°) in a universal testing machine until fracture. The specimens were subjected to a compressive load at a crosshead speed of 0.5 mm/min. The load was applied on the palatal surface at 135 degrees to the long axis of the teeth, the fracture strength values were verified in newton (N). (22)

(7) Statistical analysis

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 20. Numerical data were summarized using mean, standard deviation, and confidence interval. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups with respect to non-normally distributed numeric variables (non-parametric) were performed by the Kruskal Wallis test, followed by the Mann Whitney U test for pairwise comparison. All p-values are two-sided. P-values ≤0.05 were considered significant.

RESULTS

The highest median value was recorded in Group III (Negative control group that was significantly higher than Group I and Group II. Kruskal Wallis test revealed a significant difference between groups (p=0.011). Post hoc pairwise comparisons revealed no significant difference between groups I and II (p=0.207), (Table 1, Fig1)

TABLE (I). ¾ Descriptive statistics and comparison of fracture resistance of bleached endodontically treated teeth (Kruskal Wallis test)

	Median	Mean	Std. Dev	95% Confidence Interval for Mean		Min	Max	P
				Lower Bound	Upper Bound			
Group I	263.14[b]	290.34	115.16	231.13	349.55	165.30	449.57	0.011*
Group II	227.03[b]	248.94	76.47	209.62	288.25	155.63	389.14	
Group III	375.06[a]	375.05	100.77	323.24	426.86	199.52	501.95	

Significance level P≤0.05, *significant

Post hoc test: median sharing the same superscript letter are not significantly different

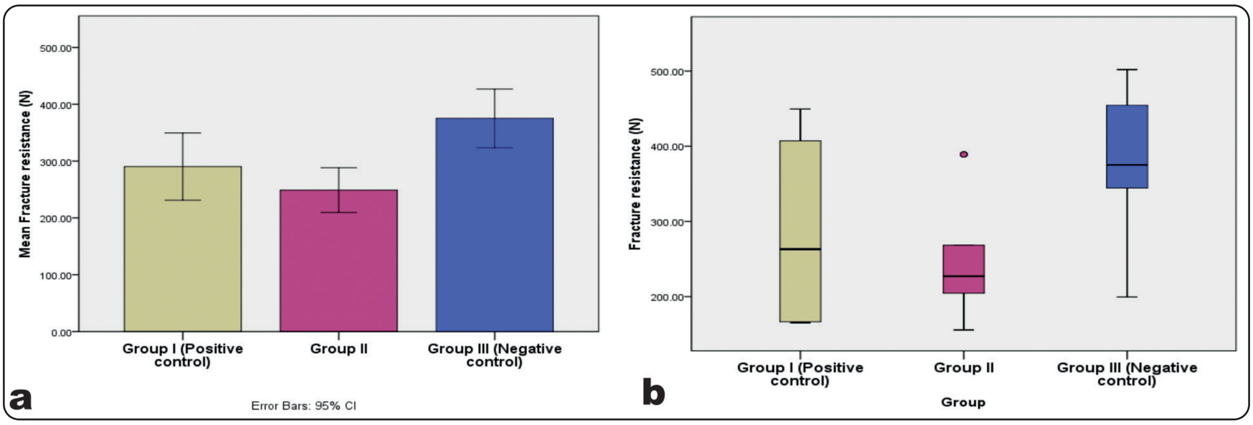

Fig. 1(a): Bar chart illustrating mean value of fracture resistance of bleached endodontically treated teeth. (b): Box plot illustrating the median value of fracture resistance of bleached endodontically treated teeth

DISCUSSION

The success rate of root canal treatment depends on many factors, among these factors are the coronal restoration of the treated teeth, the lost tooth structure during instrumentation and canal preparation, in addition to the lost water content that can render a compromised tooth structure prone to fracture. [23]

For aesthetic purposes, endodontically- treated teeth is commonly bleached intracoronally. Therefore, the fracture resistance of an endodontically-treated tooth exposed to bleaching should not be neglected.

Most investigations are concerned with the effects of PDT in terms of reduction of bacterial load. Thus, the use of PDT as a bleaching protocol and its effect on tooth structure is still scarce in literature. For this reason, this in vitro study was conducted to assess the fracture resistance of the bleached teeth. [15, 24]

In fracture resistance test, the applied force with an angle of incidence of 135° relative to the long axis of the root simulated the natural conditions in the oral cavity. [25]

The bleaching protocols (group I and II) in this study resulted in a reduction of the fracture resistance of the teeth, in comparison to unbleached teeth (group III), therefore, the null hypothesis of the study was rejected.

The significant fracture resistance reduction presented by Group I(SP) can be attributed to the presence of HP in the bleaching agents where the catalysis releases free radicals that combine with hydroxyapatite and produce apatite peroxide, these products degrade calcium and phosphate, collagen fibre and hyaluronic acid in the structure modifying the properties of the teeth. [26]

The results disagreed with the study conducted by *Praddep et al*. 2013 [27] which reflected that the fracture resistance of teeth bleached with peroxides is comparable to that of sound teeth when an axial force is applied. However, the contradiction may be due to the different testing conditions as the angle of incidence used is not consistent with clinical conditions for upper incisors.

Group II (PDT) also experienced a reduction in fracture resistance after treatment with the extract of phycocyanin for bleaching, which is considered a new protocol for bleaching that is still debatable and under investigation. [19]

PDT therapy depends on the release of a singlet oxygen that may cause alteration of the tooth structure and its properties [28]

In addition, the exact reason for fracture resistance reduction could be attributed to the difficulty in removing the photosensitizing agent from deeper regions of the pulp chamber despite the attempts done for its removal through rinsing with the aid of a plastic syringe.[19]

CONCLUSIONS

1. Bleaching of endodontically treated teeth may compromise the structural integrity of dentine and thus the coronal portion becomes more prone to fracture.
2. Bleaching using PDT decreased the fracture resistance of endodontically treated teeth.

List of abbreviations

Hydrogen peroxide	(HP)
Sodium perborate	(SP)
Carbamide peroxide	(CP)
Reactive oxygen species	(ROS)
Photodynamic therapy	(PDT)
Toluidine blue	(TB)
C-Phycocyanin	(C-PC)
Sodium hypochlorite	(NaOCl)
Ethylenediamine-tetra-acetic acid	(EDTA)
Newton	(N)

Declarations:

Ethics approval and consent to participate.

Ethics approval

The research protocol was revised and approved by the research ethics committee, faculty of dentistry, Ahram Canadian University, where the committee permitted the procedures asserted in the study.

Consent to participate.

The following study does not involve human participation.

The study was accomplished in accordance with the fundamental ethical principles and relevant guidelines.

Consent for publication

Not applicable

Availability of Data and Material (ADM)

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interest

There is no conflict of interest in this study.

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