Spectrophotometric Analysis on Bleaching Efficacy of Blood Stained Demineralized and Deproteinized Dentin – An *in vitro* Study

Ghousia S * / Sathyajith Naik N ** / VV Subba Reddy ***

Background and Objective: *The objectives of this in vitro study, is to evaluate the influence of various dentin treatment procedures prior to bleaching namely, demineralization and demineralization in conjunction with deproteinization on the dentin permeability and bleaching efficacy.* ***Method:*** *The study used a total of 40 sound premolars, which were sectioned longitudinally, and their color coefficients and absorption spectrum was recorded and used as control values for the later study. These dentin samples were then discolored by blood and their color coefficients and absorption spectrum were calibrated. They were then divided into two Groups with 20 samples each per group. Group A - dentin samples were demineralized prior to bleaching. Group B - dentin samples were deproteinized also in conjunction to demineralization prior to bleaching. The values of color coefficient and absorption spectrum were determined using Spectrophotometer for samples of each group respectively.* ***Results:*** *There were significantly higher color coefficient and absorption spectrum values in the group where dentin was treated with demineralization in conjunction with deproteinization prior to bleaching when compared to the group where dentin was treated by demineralization alone prior to bleaching.* ***Conclusion:*** *Demineralization in conjunction with deproteinization has proven to be a good method of increasing dentin permeability for achieving a higher bleaching efficacy.*

Keywords: *Sodium hypochlorite, Collagen fibrils, Demineralization, Deproteinization, Dentin permeability, Color Coefficients, Absorption Spectrum, Spectrophotometer.*
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INTRODUCTION

As patients are more desirous for whiter teeth, bleaching has become one of the most popular and common esthetic dental procedures in modern dentistry. It is a relatively easy and conservative procedure compared to other forms of treatment such as veneers and crowns.[1]

Shade change associated with root filled teeth are related primarily to improper coronal access, hemorrhage during pulpotomy, pulpectomy and trauma or because of the intracanal medicaments and filling materials remaining in the pulp chamber. The intracoronal bleaching technique was introduced by Spasser (1961), who employed a paste made of sodium perborate and water, which was temporarily placed in the pulp chamber and later modified by Nutting and Poe (1963), who replaced water by superoxol.[2]

In the past years some practitioners have added the use of acid etching during the preparation of the pulp chamber prior to bleaching procedures.[3] This step was introduced after the recognition by the profession that dentinal surfaces prepared by rotary instruments are covered by a so called "Smear layer" consisting of enamel and dentin particles, mineralized collagen matrix, blood products, and bacteria.[4]

The removal of the smear layer by acid etching has been shown to increase significantly the permeability of dentin in both vital and endodontically treated teeth by opening up the orifices of the dentinal tubules.[5] It was thus hypothesized that there is improved penetration of bleaching agents into the stained dentin, thereby enhancing their effectiveness. In the total acid etch process, the dentin surfaces are treated with etching agents that promote removal of the smear layer leading to Demineralization of dentin and also exposure of the collagen fibril network.[6]

Several authors have reported that the exposed collagen fibrils are in a destabilized and vulnerable stage to the proteolytic degradation[7-9] and have questionable durability over time. In order to avoid negative consequences related to the organic content of this tissue, the use of proteolytic substances (NaOCl or collagenase) on etched dentin can be used[10] which alters the demineralized dentin surface

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ultramorphology by dissolving the exposed collagen fibrils. The action of NaOCl promotes the exposure of a lateral runway network and amplifies the dentinal tubules,[11] rendering a dentin similar to etched enamel, which is a favorable characteristic for dentin permeability.

Although the color of an object can usually be analyzed visually or instrumentally in dentistry visual comparison with shade guides is generally employed.[12] Visual assessment has been found to be subjective, unreliable and inconsistent. Color determination is not consistent among different clinicians and can also vary for the same clinician. Assessment and consistency may be improved by the use of a spectrophotometer.[13]

Comparison of the bleaching efficacy on blood stained dentin treated differently in order to render dentin demineralized and deproteinized and to assess the efficacy and permeability of bleaching regimen will be carried out in this *in vitro* study.

METHOD AND MATERIALS

A total of 40 freshly extracted premolars from orthodontic patients aged between 12-18 years were used in the study. These 40 teeth were stored in buffered isotonic saline (pH 7.4) at 4˚C until required. The teeth were sectioned longitudinally with Low Speed Micromotor Handpiece *–NSK by Naganishi Inc. Japan* to get uniform thickness of 1mm of each sample, with a Diamond cutting disk at 1000 rpm.

Spectrophotometric Analysis of Unstained dentin samples

The samples were then placed on a white non-reflecting background and only the labial side of the coronal middle-third of dentin of $1mm^3$ with uniform thickness and density were subjected to Spectrophotometer. The color coefficients and absorption spectrum of each dentin sample was recorded.

Blood-staining of dentin samples by Centrifugation

The dentin samples were discolored by a technique, initially devised by Freccia and Peters (1982) and modified by Marin *et al* (1997) to provide satisfactory *in vitro* model to mimic the staining of a tooth following trauma and subsequent hemorrhage into the pulp chamber.

To hemolyze the red blood cells and have the breakdown products penetrate the dentinal tubules, the prepared dentin samples were then placed individually, crown first in Centrifuge tubes of a High speed bench Centrifuge (R-8C) and immersed in packed red blood cells. They were then centrifuged at 5000 rpm thrice daily for 30 minutes over three consecutive days.

In the interim, the teeth will be stored at 37˚C and 100% humidity.

Spectrophotometric Analysis of Blood stained dentin samples

The stained dentin samples were removed at random from each of the centrifuge tubes and were blotted on a damp filter paper to remove excess blood but were left moist. These samples were then subjected, as the earlier dentin samples against a white non-reflecting background, that is, only the labial side of the coronal middle-third of dentin of $1mm^3$ with uniform thickness and density were subjected to Spectrophotometer and the color coefficients and absorption spectrum of each blood-stained dentin sample was then recorded.

Further, these blood-stained dentin samples were divided into 2 groups.

Distribution of blood stained samples

Random division of 40 dentin samples was done into 2 groups :

Group A: 20 blood-stained dentin samples were used to render dentin Demineralized.(DM)

Group B: 20 blood-stained dentin samples were used to render dentin Demineralized + Deproteinized. (DMP)

Sample Preparation

Group A samples for Demineralization (DM) procedure

The blood stained dentin samples were treated with 37% orthophosphoric acid for 15 seconds with an applicator tip, washed thoroughly and then air dried for 20 seconds.

Group B samples for Demineralization+ Deproteinization (DMP) procedure

The blood stained dentin samples were treated with 37% orthophosphoric acid for 15 seconds with an applicator tip, washed thoroughly and then air dried for 20 seconds. These samples were then treated by immersion in 5% sodium hypochlorite solution for 5 minutes.

Bleaching procedure

Following the procedure of dentin DM and DMP, the Group A and Group B samples were blotted on filter paper and then subjected to immersion in Bleaching regimen (30% H_2O_2 & Sodium perborate) for 5 min, and then blotted on filter paper.

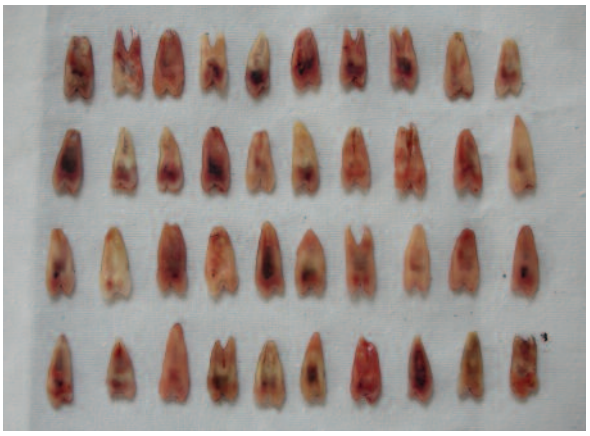

Figure 1. Sectioned samples Stained with Blood

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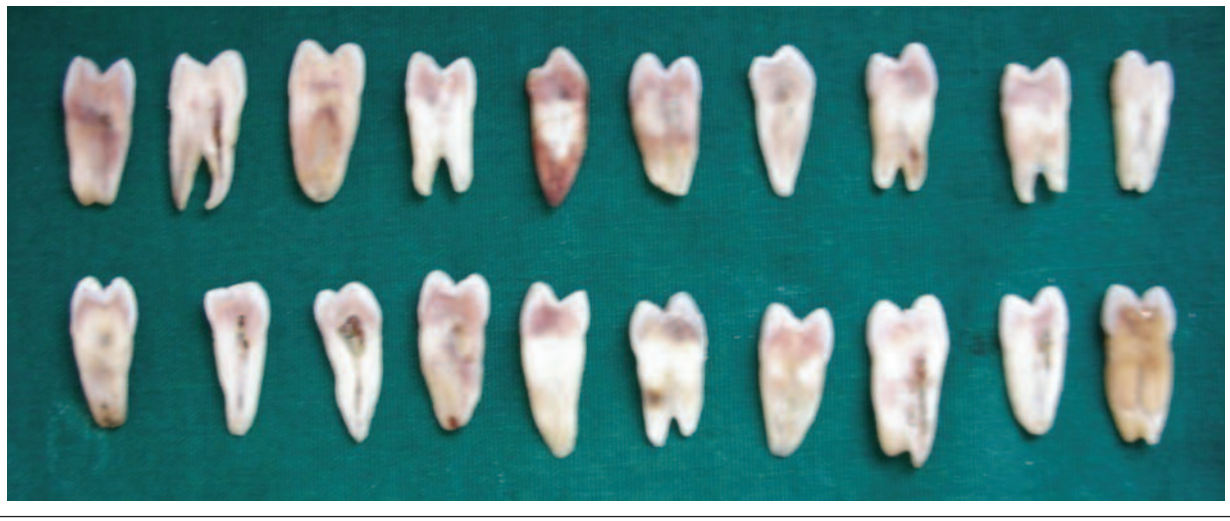

Figure 2. Bleached samples after Demineralization

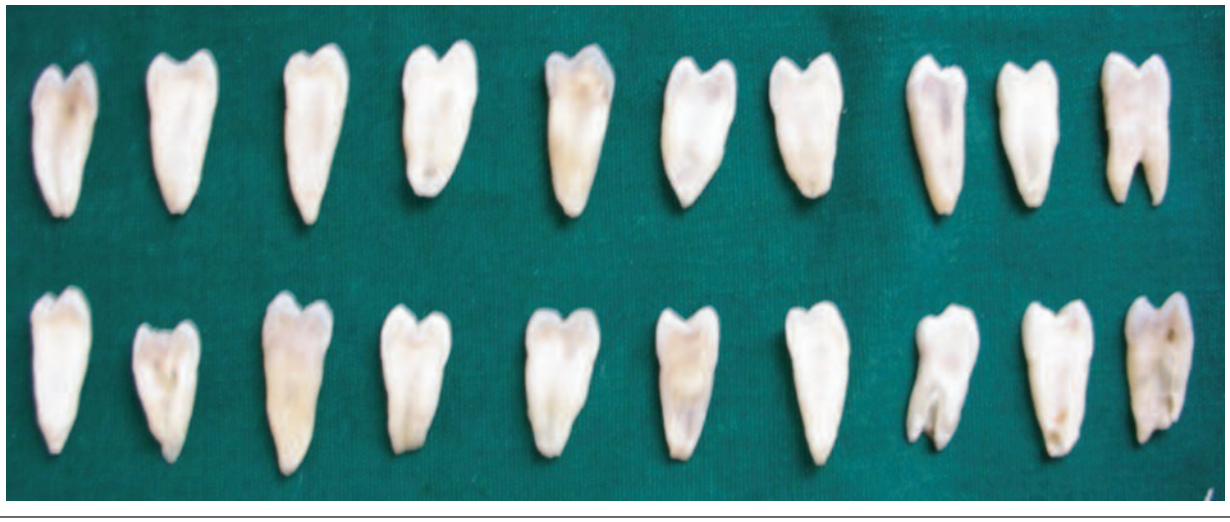

Figure 3. Bleached samples after Demineralization + Deproteinization

Spectrophotometric analysis of bleaching efficacy

Group A and Group B samples were then placed on a non-reflecting white background blocks and only the labial side of the coronal middle-third of dentin of 1mm^3 with uniform thickness and density were subjected to Spectrophotometric (Minolta CM-330 1d) analysis.

The color coefficients and absorption spectrum of each dentin sample was analyzed by Jaypak 4808 Quality control system software and recorded separately for Group A and Group B samples.

The CIE (Commission Internationale de I'Eclairage) Lab, (Vienna, Austria) (Westland 2003) color coordinates system which provides information about location of object color in a uniform 3-D color space was used in this study to determine color coefficients and absorption spectrum of the dentin samples against a white background.

The Absorption Spectrum of each dentin sample was assessed separately by recording the reflectance percentage in the visible spectral region ranging from 360nm to 780nm at an interval of 10nm at each wavelength. The measurements in a spectral range of 560nm wavelength was taken for each sample in this study and recorded. The data was then submitted to statistical analysis.

RESULTS

Descriptive statistics comprised calculation of Mean and Standard Deviation followed by Least Significant Difference (LSD) / Analysis of Variance for repeated measurements.

One-Way ANOVA test was applied for evaluating the bleaching efficacy of both the groups, such as Blood-stained dentin samples followed by Demineralization procedure with that of Blood-stained dentin samples followed by Demineralization+Deproteinization(DMP) procedure to derive at significant comparison.

Table 1: Illustrates the Color Coefficient of mean in different set of samples of different groups

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Table 1. Comparison of color coefficients observed in different samples

	Mean Values		P* Value	Significance
	DEMINERALIZED (GROUP C)	DEPROTEINIZED (GROUP D)		
Difference with Unstained	-0.0365	-0.0345	P>0.05	NS
Difference with Blood-Stained	0.027	0.0205	P>0.05	NS

Table 2. Comparison of absorption spectrum observed in different samples (560 nm visible range)

	Mean Values		P* Value	Significance
	DEMINERALIZED (GROUP C)	DEPROTEINIZED (GROUP D)		
Difference with Unstained	11.03	5.35	P<0.05	S
Difference with Blood-Stained	-8.85	-14.4	P<0.05	S

Table 2: Illustrates the Absorption Spectrum of mean in different set of samples of different groups.

Graph 1: Illustrates the significant difference of Group C and Group D in Unstained samples was seen to exhibit P value of <0.05. There was a statistically significant difference between Group C and Group D.

Graph 2: Illustrates the significant difference of Group C and Group D in Stained samples was seen to exhibit P value of <0.05. There was a statistically significant difference between Group C and Group D.

DISCUSSION

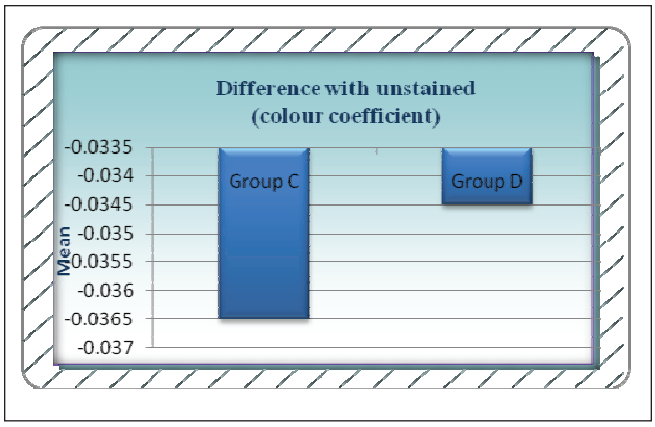
It has been considered that the depth of penetration by blood determined the degree of tooth discoloration. If only the pulp chamber was discolored, the tooth would appear lusterless and dull grey in color. However, if the hemoglobin penetrated deeply into the dentinal tubules, the crown would be grayish-black or grayish-brown in color.[14] Therefore, in the present study, the teeth samples were submitted to human blood in order to simulate tooth discoloration caused by blood pigments.[2]

A technique devised by Freccia and Peters[15] used to produce blood-stained teeth did not reproduce *in vivo* staining caused by trauma and subsequent hemorrhage into the tooth because in this technique the whole tooth is immersed in blood, which may allow staining to occur from an external surface rather than from pulp chamber alone. Therefore, a modified version developed by Marin *et al* (1992) was used in this study to provide a satisfactory *in vitro* model to mimic the staining of a tooth following trauma and subsequent hemorrhage into pulp chamber,[16] which is accomplished by sectioning the specimens .

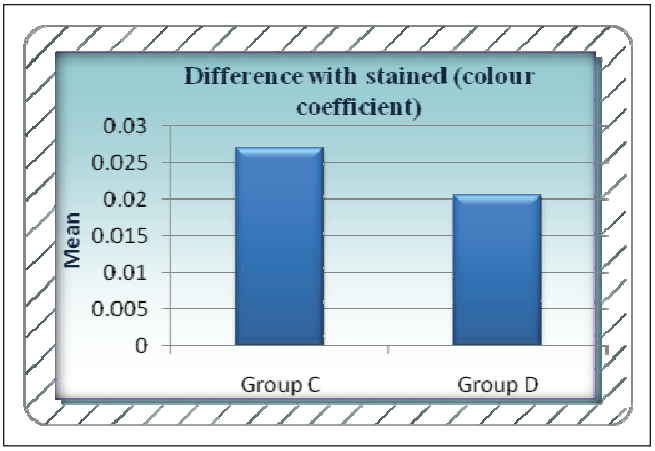
The rationale for staining the samples was to allow for a more discriminative comparison of the differently treated dentin rendering DM and DMP followed by a bleaching regimen and in particular, the evaluation of in depth (bleaching of dentin) action.

One of the most important properties of a bleaching material is its ability to allow penetration of the bleaching agent through dentinal permeability.[17] The deeper the penetration, the more pigment that causes chromatic alteration of the dental tissues can be reversed by the oxidation reaction.[18]

Howell RA in the year 1980, recommended the acid etching of dentin with 30% phosphoric acid prior to intra-coronal bleaching in order to open up the dentinal tubules and enhance penetration by the bleaching agent.[19] This acid-etching procedure promotes the removal of smear layer, exposure of collagen fibril network and renders dentin demineralized



Graph 1. Illustrating difference in means of color co-efficient of samples of different groups followed by bleaching with that of unstained group



Graph 2. Illustrating difference in means of color co-efficient of samples of different groups followed by bleaching with that of stained group

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increasing its permeability.[6] Further, Hansen and Asmussen in the year 1997, proved that the etching of dentin removes hydroxyapatite, leaving collagen fibers without support except for that caused by the water contained within dentin.[20] In order to avoid negative consequences related to the organic content of this tissue, the use of proteolytic substances on etched dentin has been suggested by Vargas *et al*.[10]

As *per* the findings of the present study, it was observed that, the blood stained teeth samples when treated by DM procedure did not as significantly reduce the discoloration of the samples when compared to the reduction of blood discoloration when treated by DMP procedure with the value P>0.05 for color co-efficient recordings, and with the value P<0.05 for absorption spectrum recordings in both the groups.

The color coefficient values of samples which were bleached after DMP procedure showed values closer to that of Unstained samples, that is the readings were similar to that as prior to blood-staining when compared to the samples which were bleached after DM procedure only. The difference between them exhibited a P value of >0.05. The Absorption Spectrum values also showed that the samples bleached after DMP procedure showed was more similar to the values of unstained samples with a P value of < 0.05.

The probable result of the darker shade and better bleaching efficacy could be attributed to increased permeability of dentinal tubules and depletion of unstable collagen fibers exposed after DM of dentin, thus altering the dentin surface and modifying hydrophilic properties, attributing to the amplification of opening up of dentinal tubules.

The use of deproteinizing solutions (NaOCl or collagenase) alters the demineralized dentin surface ultramorphology by dissolving the exposed collagen fibrils and promotes the exposure of a lateral runway network and amplifies the dentinal tubules,[11] rendering a dentin similar to etched enamel, which is a favorable characteristic for dentin permeability.[21] The dissolution of organic tissues by NaOCl solutions is based on the action of chloride over the proteins, forming chloramines, which are soluble in water. This reaction is directly proportional to the active chloride concentration present in the solution.[22]

NaOCl solution alters the configuration or removes the organic components of dentin; especially, the collagen fibrils[23] rendering dentin Deproteinized. The Deproteinized dentin has higher hardness, modulus of elasticity,[24] wettability,[25] and permeability[26] than the Demineralized dentin. The dentin substrate is transformed, after DP, in a very porous structure with multiples irregularities and anastomoses, which could not be seen only by the normal DM process.[27]

It is also observed that the tubular diameter on Deproteinized substrate was increased and had funnel configuration. Deproteinization (DP) produced a complex ultramorphological pattern of the dentin that could be considered a transition between the acid etched and the intact dentin substrate.[23]

In the present study, the Spectrophotometer Minolta CM-330 1d, was used which emitted within a reference tooth and the reflected light is decomposed into its spectral components by diffraction and compared with the incident light (Baltzer and Kaufmann-Jinoian 2005); thus, Spectrophotometers provide highly accurate measurement of absolute shades.[28] The color parameters in the present study were recorded in the L* a* b* color space, as established by the CIE in 1976.[29]

Caution must be exercised when applying the results of this *in vitro* study to clinical conditions.

CONCLUSION

It can be concluded that Demineralization (DM) in conjunction with Deproteinization (DP) has proven to be a good method of increasing dentin permeability for achieving a higher bleaching efficacy with better color coefficients and absorption spectrum through Spectrophotometric analysis.

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