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Research Article

THE TROJAN BATTLE FOR COLLAGEN

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ABSTRACT

The aim to determine the collagenolytic activity following bleaching in non- vital teeth and methods employed to counteract it using FT-Raman spectroscopy. 25 extracted non- carious human third molars were obtained. The teeth were stored in thymol solution at 4˚C and used within 1 week after extraction. Dentin disks were obtained from the mid-coronal portion of each tooth, 50 dentin beams were obtained. They were divided into five groups (n=10) and subjected to the following treatment Group A: Untreated dentin. Group B: Dentin disks bleached in 35% hydrogen peroxide.Group C:10% sodium ascorbate was applied on bleached dentin disks. Group D:0.2% chlorhexidine digluconate was applied on bleached dentin disks. Group E:10% sodium ascorbate followed by 0.2 chlorhexidine digluconate was applied on bleached dentin disks. The collagenolytic changes were observed using Raman transform infrared spectroscopy. Concluded that use an antioxidant & a MMP inhibitor to preserve the structural integrity of the collagen following bleaching.

INTRODUCTION

Bleaching is one of the most documented clinical techniques in dentistry and performed for over a century. The current trend toward cosmetic dentistry and the growing demands of the patients for beautiful white teeth, the bleaching of nonvital teeth has become increasingly important in recent years.

Extrinsic discolouration occurs when some agent literally stains or damages the enamel surfaces of the teeth. They are found on the outer surface of teeth and are usually of local origin which can be removed by oral prophylaxis. Cigarettes, cigars and pipes will produce a yellowish brown to black discolouration, usually in the cervical portion of the teeth and primarily on the lingual surfaces. Chewing tobacco frequently penetrate the enamel producing a deeper stain. Coffee and tea cause severe tenacious discolorations, usually brown to black stain.

Intrinsic discolouration, are stains within the enamel and dentin caused by the deposition or incorporation of substances within these structures, such as Tetracycline stains, Dentinogenesis imperfecta, Fluorosis byproducts released into the dentinal tubules during illness (e.g., bilirubin involved with jaundice), Trauma (primarily the breakdown of haemoglobin), or pigmentation escaped from the medicaments and materials used in restorative dentistry.

In the middle of the 19th century, first attempt was made to lighten discolored teeth using various agents. Initially, oxalic acid was used, until the bleaching action of hydrogen peroxide was discovered in 1884 (Goldstein 1997). Bleaching of nonvital teeth was first mentioned by Garretson in 1895, who used chlorine as the bleaching agent (Fasanaro 1992). However, it wasn't until 1951 that hydrogen peroxide was used to bleach nonvital teeth (Pearson 1951). The bleaching of nonvital teeth is a minimally invasive procedure which when performed correctly, bears only slight risks.

The three most popular techniques for nonvital tooth bleaching are the walking bleach technique, inside/outside bleaching, and in-office bleaching. The walking bleach technique is a relatively reliable, fairly simple technique for dentists and patients. Inside/outside bleaching can be used additionally when internal and external bleaching must be combined. In-office bleaching seems to be a short-term solution, the effects of which can largely be attributed to dehydration of the teeth. In Thermocatalytic technique, insufficient cervical sealing, and high concentrations of bleaching agents should be avoided, as this can increase the risk of cervical root resorption. Patients should be informed about the low predictability of bleaching success and the risk of recurrent discoloration.

Hydrogen peroxide, in various concentrations, is the primary material currently used in the bleaching process. The mechanism of action of H2O2 in tooth bleaching is considered to be oxidation, although the process is not well understood. It is advocated that the oxidizers remove some unattached organic

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matter from the tooth without dissolving the enamel matrix but also may change the discolored portion to a colorless state.

Two of the key factors in determining the overall tooth whitening efficacy of peroxide-containing bleaching agents are the concentration of the peroxide and duration and number of times of application. Dentin matrix metalloproteinases (MMPs) -2, -8, -9, and -20 are structural endopeptidases that contribute to dentin matrix organization and mineralization.C-terminal telopeptide of type I collagen(ICTP) is the carboxyterminal telopeptide of type I collagen that is joined via trivalent cross-links and liberated during collagen degradation. The telopeptide is only produced through the action of MMPs, and it is considered an index of MMP-driven collagenolysis and an augmented collagenolytic activity of MMPs causes an increased production of reactive oxygen species. So we aimed to ascertain the effect of hydrogen peroxide bleaching agent on MMP-mediated dentin collagen degradation.

It was observed that the use of anti-oxidant agents before the bonding process can reverse the compromised bonding to bleached enamel. Thus, the restorative procedures could be performed immediately after the bleaching procedures, thereby reducing the total time of the complete esthetic treatment. The ascorbic acid is a reducing agent capable to donate two high-energy electrons to scavenge the free radicals. The application of 10% sodium ascorbate was effective to reverse the compromised bonding to oxidized dentin and enamel. The use of the sodium ascorbate, instead of the ascorbic acid, is recommended to avoid the potential double-etching effect of this mild acid on etched teeth.

Chlorhexidine has been widely used as an antimicrobial agent, including for disinfection before the placement of restorations. The most plausible explanation by Gendron *et al*.,(1999) is that chlorhexidine causes inhibition of dentin matrix-bound MMPs resulting in decreased degradation of collagen fibrils resulting in decreased degradation of hybrid layer and sub-hybrid layer collagen fibrils..Optimal concentration and time of application of chlorhexidine are mandatory for MMPs inhibition that would result in the best time-related preservation of the dentin-adhesive interface.

Hence the aim of this in- vitro study was to determine the collagenolytic activity following bleaching in non- vital teeth and methods employed to counteract it using FT-Raman spectroscopy.

MATERIALS AND METHODS

Twenty five extracted non- carious human third molars were obtained with informed consent from different donors under a protocol approved by the institution review board. The teeth were stored in 0.1% (w/v) thymol solution at 4˚C and used within 1 week after extraction. Dentin disks (0.75- 0.08 mm thick) were obtained from the mid-coronal portion of each tooth by using a slow-speed diamond saw (IsoMet; Buehler Ltd, Lake Bluff, IL) under water cooling. Two dentin beams were obtained from each dentin disk as previously described by Osorio *et al* . A total of 50 dentin beams were obtained.
They were divided into five groups (n=10) and subjected to the following treatment

Group A: Untreated dentin.
Group B: Dentin disks bleached in 35% hydrogen peroxide.

Group C: 10% sodium ascorbate was applied on bleached dentin disks.

Group D: 0.2% chlorhexidinedigluconate was applied on bleached dentin disks.

Group E: 10% sodium ascorbate followed by 0.2 chlorhexidinedigluconate was applied on bleached dentin disks.

The collagenolytic changes in the dentin specimens were observed using Raman transform infrared spectroscopy.

RESULTS

Collagenolytic activity was maximum in group B followed by group E,C & D respectively.

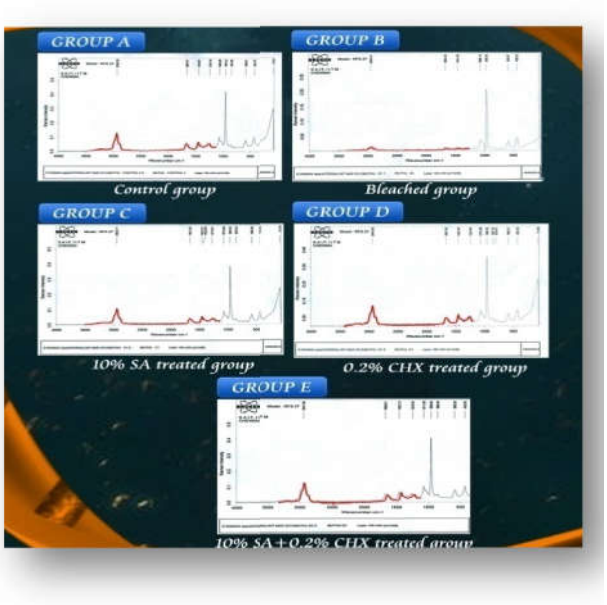

Table 1 Collagenolytic activity of Group A,B,C,D,E by using FT-Raman spectroscopy

DISCUSSION

Tooth discoloration varies in etiology, appearance, localization, severity, and adherence to tooth structure. It may be classified as intrinsic, extrinsic, and a combination of both (Hattab*et al.*, 1999). Intrinsic discoloration is caused by incorporation of chromatogenic material into dentin and enamel during odontogenesis or after eruption. Exposure to high levels of fluoride, tetracycline administration, inherited developmental disorders, and trauma to the developing tooth may result in pre-eruptive discoloration. After eruption of the tooth, aging, pulp necrosis, and iatrogenesis are the main causes of intrinsic discoloration. Coffee, tea, red wine, carrots, oranges, and tobacco give rise to extrinsic stain (Hattab*et al.*, 1999; Watts and Addy, 2001).

Intracoronal bleaching is a conservative alternative to the more invasive esthetic treatment of non-vital discolored teeth.Careful examination is necessary, since the method requires healthy periodontal tissues and a root canal that is properly obturated to prevent the bleaching agent from reaching the periapical tissues (Baratieri*et al.*, 1995).

Contemporary tooth whitening processes involve the use of either hydrogen peroxide or carbamide peroxide. Discolorations arise due to the formation of chemically stable,

chromogenic products. Pigments consist of long-chain organic molecules. In bleaching, these compounds are oxidized: they are split into smaller molecules which are usually lighter. During bleaching, the long-chain organic molecules are transformed into carbon and water, and-together with nascent oxygen-are released.

FTIR analysis revealed that H2O2 treatment induces the loss of both carbonate and proteins from enamel and dentin. Substitutions in the hydroxyapatite mineral crystal by carbonate increase mineral solubility at low pH (Taube *et al*., 2010). Indeed, the loss of carbonate in enamel and dentin relates to the acidity of 35% H2O2, whereas changes in enamel and dentin organic content relate to protein loss, especially collagen in dentin, by the strong oxidizing ability of peroxide.

Acidity of bleaching agents can trigger the autocatalytic activation of MMPs and stabilization of cysteine proteases present in the dentin-pulp complex as suggested by Tersariol *et al*., 2010 and Nascimento *et al.*, 2011. It was Buck *et al*., in 1992 who stated that Cathepsin B is also able to degrade purified extracellular-matrix proteins under both acidic and neutral pH. Also, cysteine cathepsins are able to activate MMPs. Analysis of our data strongly suggests that both classes of proteolytic enzymes, cysteine cathepsins and MMP, are activated in mineralized dentin during degradation during tooth-whitening treatment with 35% H2O2.

Many studies consider a 10% sodium ascorbate (SA) solution to be an effective choice to improve dentin bonding agent and defend the use of SA as the most efficient agent for neutralizing the oxidizing effects of bleaching agents. Its use may also increase the strength of dentin bond. The antioxidant type, concentration, form, and duration of application have been considered important factors for improving bonding after bleaching treatment.

The decrease in bond strength and reduction of the adhesive system interaction in bleached teeth have attributed to the presence of residual oxygen. This highly reactive chemical element eliminates pigmentation but also reacts with the free radicals of resin materials which inhibits polymerization and generate polymers with reduced mechanical properties.

Histochemical studies have shown reduced levels of sulfur. Sulfur is a component of proteoglycans (chondroitin sulfate and keratin sulfate), and changes in their levels indicate damage to the organic dentin matrix. These changes in proteoglycans might interfere in the maintenance of interfibrillar spaces, which may have compromised diffusion of the adhesive into the collagen network, potentially reducing the bond strength.

Despite reports that the use of bleaching agents at low concentrations has been considered absolutely safe, analysis of our data shows that the use of 35% H2O2 as a bleaching agent provokes biological responses of dental tissues that can be clinically adverse in the long term and/or after recurring bleaching treatments. In contrast, Goo *et al*.(2004) demonstrated that mineral loss caused by dental bleaching was not a threat to teeth. In addition, Lee *et al*. (2006) showed that the amount of calcium lost from teeth after 12 h of bleaching treatment was similar to that lost from teeth exposed to a soft drink or juice for a few minutes.

The application of an antioxidant or MMP inhibitor would not be efficient in preventing collagen degradation. In this study, the application of an antioxidant followed by a MMP inhibitor on bleached dentin disks showed the least collagen degradation, indicating the stability of collagen.

CONCLUSION

Within the limitations of this study, it is mandatory to use an antioxidant & a MMP inhibitor to preserve the structural integrity of the collagen following bleaching. Further studies are required to determine the effects of 10% sodium ascorbate & 0.2% CHX in the polymerization process of adhesive material following bleaching.

References

1. Manuel Toledano, Monica Yamauti, Estrella Osorio, and Raquel Osorio, Bleaching Agents Increase Metalloproteinases-mediated Collagen Degradation in Dentin, JOE-Volume 37, Number 12, December 2011
2. Ellias E, Sajjan G, Effect of bleaching on microleakage of resin composite restorations in non-vital teeth:An in-vitro study, *Endodontology*, Vol. 14, 2002
3. Dr. Brigitte Zimmerli, Klinik für Zahnerhaltung, Präventiv- und Kinderzahnmedizin, Bleaching of Nonvital Teeth A Clinically Relevant Literature Review, Schweiz Monatsschr Zahnmed Vol. 120 4/2010
4. C.K.Y. Yiu1, F. García-Godoy2, F.R. Tay1*,D.H. Pashley3, S. Imazato4, N.M. King1, and S.C.N.Lai1, A Nanoleakage Perspective on Bonding to Oxidized Dentin, *J Dent Res* 81(9) 2002
5. Nataya Vongphan*, Pisol Senawongse1, Waraporn Somsiri2, Choltacha Harnirattisai1, Effects of sodium ascorbate on microtensile bond strength of total-etching adhesive system to NaOCl treated dentine, N. Vongphan *et al*.
6. Carlos Rocha Gomes Torres, Alexandre Fuzuko Koga*, Alessandra Bühler Borges, The effects of anti-oxidant agents as neutralizers of bleaching agents on enamel bond strength**,** *Braz J Oral Sci. 5(16):971-976*
7. K. Castro Æ M. Pe´rez-Alonso Æ M. D. Rodrı´guez-Laso, L. A. Ferna´ ndez Æ J. M. Madariaga, On-line FT-Raman and dispersive Raman spectra database of artists' materials (e-VISART database)
8. B. Schrader a,*, H. Schulz b, G.N. Andreev c, H.H. Klump d, J. Sawatzki e, Non-destructive NIR-FT-Raman spectroscopy of plant and animal tissues, of food and works of art, *B. Schrader et al*. : *Talanta* 53 (2000) 35-45
9. Marena gasior-glogowska, Malgorzata komorowska, Jerzy hanuza, Maciej ptak, Magdalena kobielarz, Structural alteration of collagen fibres- spectroscopic and mechanical studies, M.Gasior-glogowska *et al*., *Acta of Bioengineering and Biomechanics Vol. 12, No. 4, 2010*
10. M.Miyasaki, H.Onose, B.K.Moore, Anaysis of the dentin- resin interface by use of laser raman spectroscopy, M.Miyasaki *et al* ./ Dental materials 18(2002)576-580
11. Shuhei Tsuchiya ,1,2 James P Simmer,1 Jan C-C Hu,1 Amelia S Richardson,1 Fumiko Yamakoshi,1 and Yasuo Yamakoshi1, Astacin Proteases Cleave Dentin

Sialophosphoprotein (Dspp) to Generate Dentin Phosphoprotein (Dpp), TSUCHIYA *et al*.

12. Corinne Gullekson, Leanne Lucas, Kevin Hewitt, and Laurent Kreplak*, Surface-Sensitive Raman Spectroscopy of Collagen I Fibrils, *Biophysical Journal* Volume 100 April 2011 1837-1845
13. N. Laila Huq a, Ashwini Loganathan a, Keith J. Cross a, Yu-Yen Chen a, Neil I. Johnson b, Matthew Willetts b, Paul D. Veith a, Eric C. Reynolds a, Association of bovine dentine phosphophoryn with collagen fragments, Archives of Oral Biology (2005) 50, 807-819
14. Luciano Bachmanna,*, Rolf Diebolderb,1, Raimund Hibstb,1, Denise Maria Zezella, Changes in chemical composition and collagen structure of dentine tissue after erbium laser irradiation, *L. Bachmann et al. / Spectrochimica Acta Part A 61 (2005) 2634-2639*
15. R. Lemor,1 M. B. Kruger,1 D. M. Wieliczka,1* P. Spencer2 and T. May3, Dentin etch chemistry investigated by Raman and infrared spectroscopy**,** R. LEMOR *ET AL*., *J. Raman Spectrosc*. 31, 171-176 (2000)
16. Kunio Ikemuraa, Franklin R. Tayb, Toshiya Hironakac, Takeshi Endod, David H. Pashleye,*, Bonding mechanism and ultrastructural interfacial analysis of a single-step adhesive to dentin, K. Ikemura *et al*. / *Dental Materials* 19 (2003) 707-715
17. Mitsuo Satoa, Masashi Miyazakib, Comparison of depth of dentin etching and resin infiltration with single-step adhesive systems, *Journal of Dentistry* (2005) 33, 475-484
18. M. R. O. Carrilho, R. M. Carvalho, M. F. de Goes, V. di Hipólito, S. Geraldeli, F. R. Tay, D. H. Pashley4, and L. Tjäderhane5, Chlorhexidine Preserves Dentin Bond in vitro, *J Dent Res*. 2007 January ; 86(1): 90-94.
19. Atul Jain, Rachana Bahuguna, Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview, *journal of oral biology and craniofacial research* 5 (2015) 212-218
20. Andrew Joiner, The bleaching of teeth: A review of the literature, *Journal of dentistry* 34 (2006) 412-419

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