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ORIGINAL RESEARCH

Effect of Photo-Fenton Bleaching on Tetracycline-stained Dentin *in vitro*

[1]Zackary Yale Bennett, [2]Laurence James Walsh

ABSTRACT

Aim: Tetracycline-stained tooth structure is difficult to bleach using nightguard tray methods. The possible benefits of in-office light-accelerated bleaching systems based on the photo-Fenton reaction are of interest as possible adjunctive treatments. This study was a proof of concept for possible benefits of this approach, using dentine slabs from human tooth roots stained in a reproducible manner with the tetracycline antibiotic demeclocycline hydrochloride.

Materials and methods: Color changes overtime in tetracycline stained roots from single rooted teeth treated using gel (Zoom! WhiteSpeed®) alone, blue LED light alone, or gel plus light in combination were tracked using standardized digital photography. Controls received no treatment. Changes in color channel data were tracked overtime, for each treatment group (N = 20 per group).

Results: Dentin was lighter after bleaching, with significant improvements in the dentin color for the blue channel (yellow shade) followed by the green channel and luminosity. The greatest changes occurred with gel activated by light ($p < 0.0001$), which was superior to effects seen with gel alone. Use of the light alone did not significantly alter shade.

Conclusion: This proof of concept study demonstrates that bleaching using the photo-Fenton chemistry is capable of lightening tetracycline-stained dentine. Further investigation of the use of this method for treating tetracycline-stained teeth in clinical settings appears warranted.

Clinical significance: Because tetracycline staining may respond to bleaching treatments based on the photo-Fenton reaction, systems, such as Zoom! WhiteSpeed, may have benefits as adjuncts to home bleaching for patients with tetracycline-staining.

Keywords: Tetracycline, Staining, Bleaching, Intense light, Laboratory study.

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[1,2]School of Dentistry, The University of Queensland, Brisbane Australia

Corresponding Author: Laurence James Walsh, Professor School of Dentistry, The University of Queensland, Brisbane Australia, Phone: 61733658160, e-mail: l.walsh@uq.edu.au

INTRODUCTION

Local or systemic exposure to tetracyclines can result in staining of tooth structure. This problem both limits systemic administration of tetracycline antibiotics to periods other than during anterior tooth formation,[1-3] and raises concerns of iatrogenic staining for certain endodontic medicaments and irrigants.[4-10] Bleaching may be a more conservative option for affected teeth than full coverage crowns or veneers.[11-13] While nightguard vital bleaching can be effective, prolonged treatment times are needed.[14-17] There is interest in the concept of in-office power bleaching to augment or substitute for nightguard bleaching. Past clinical studies have shown photo-oxidation of developmental tetracycline stains in vital teeth by in-office photodynamic bleaching methods using intense visible light from KTP lasers when combined with an alkaline hydrogen peroxide gel, indicating a place for office-based bleaching methods in the treatment of this condition.[13]

Given the high cost of KTP laser bleaching treatments, there is value in exploring the benefits of more common and less expensive in-office bleaching systems, particularly those which use visible light to accelerate bleaching by photochemical reactions. The Zoom! WhiteSpeed® system is a typical example of the photo-Fenton chemistry used in dental bleaching. This employs 25% hydrogen peroxide in a gel which is then irradiated with intense visible blue light from an LED array.[18,19] While such systems are used commonly for treating age-related tooth discoloration from dentin sclerosis, it is unclear whether they can provide benefits for more difficult forms of tooth discoloration, such as those caused by tetracyclines. As a first step in exploring this possibility, the present study used an established model for tetracycline staining of human dentin, to assess the potential usefulness of this system, comparing the effects of the gel and the irradiating LED lamp alone and when used in combination. While not a direct analog of the clinical situation, the advantages of this model are that it provides a reproducible level of darkly stained dentin with a known type of tetracycline and with defined exposure parameters, and allows comparison of adjacent slices of dentin from the same tooth when treated with different methods, thus removing many confounders which operate in clinical studies.

MATERIALS AND METHODS

The roots canals of extracted human teeth which had undergone routine preparation with rotary nickel titanium files were treated with a commercial tetracycline (demeclocycline hydrochloride) paste (Ledermix®, Lederle Laboratories, Wolfratshausen, Germany) for 12 weeks to induce discoloration, as previously described in detail.[8] The teeth were collected with approval of the institutional ethics committee. A total of 80 root slices were prepared by sectioning the roots into at least three separate 2 to 3 mm thick horizontal slices, from the crown down, using a fine diamond saw with distilled water irrigation. These thin slices were then divided in half using diamond disks. A bur mark was placed to indicate orientation. Slices from the same root were distributed equally across all four experimental groups (LED light alone for 30 minutes, gel alone for 30 minutes, gel plus light (as recommended by the manufacturer), or untreated controls). To remove smear layer from the sectioning procedure before bleaching treatments, dentin slices were immersed in orthophosphoric acid (470 gm/l) for 1 minute, before being rinsed.

Baseline images were recorded of the coronal aspect of each slice using a 4 megapixel Nikon Coolpix digital camera (Nikon, Tokyo, Japan) attached to a microscope (25× magnification) with fixed exposure settings (F3.3 for 0.5 second). Standardized lighting and magnification were used with the lighting positioned to minimize reflections. Each slice was stored immersed in distilled water in separate wells of a 96-well immunoassay plate in a moist airtight container before and after treatment. Images were recorded immediately following treatment with Zoom! Whitespeed® for 30 minutes using the recommended methods for applying gel and light specified by the manufacturer (Philips Discus, Culver City, CA). Samples were photographed again after 1 week, and after 1 month following treatment. Each set of images included a color calibration card, which provided an internal control to verify that exposure parameters were identical overtime. Pre- and post-treatment images were checked for standardization of the color card to ensure that luminosity values for all six color squares on the color card were within 5% of one other.

After removing all the components of the images other than the dentin slice (including removing any reflections of the illuminating light source) using the magic wand tool in GIMP® version 2.8.3 software, the histogram analysis function was used to calculate the mean red, green, blue, and luminosity levels from the pixel data across the whole dentin slice. This method gave in the order of 5 to 600,000 pixels per slice from which color channel information could be derived for each of the four channels. The reasons for using this parti-cular approach are that is objective and allows for fine grained discrimination of changes in both the red and green hues as separate variables, which is highly relevant as tetracycline-stained teeth change during bleaching from brown to yellow. All data sets displayed a normal distribution, allowing for parametric statistical analyses. Changes overtime in color channel data were assessed using repeated measures ANOVA with Tukey-Kramer post-hoc tests.

RESULTS

An assessment of variation between individual untreated slices at baseline did not show significant variation between groups in any of the 4 channels (red, green, blue, luminosity), confirming the consistency of the samples at baseline and the equivalency of the groups before treatment.

Bleaching gel plus light provided a significant lightening effect which was evident immediately after treatment. The magnitude of this effect was greatest in the blue channel (giving a reduction in the yellow color) ($p < 0.0001$, mean difference 39.5, CI 28.0-51.1), and progressively lesser in green, red and luminosity channels (Figs 1 and 2). Bleaching gel activated by visible blue light gave significantly greater effects than gel alone without light ($p = 0.0002$; mean difference 25.8, CI 13.3-39.2). Light when used alone without bleaching gel did not cause a significant change compared with baseline or with untreated controls, and as such also did not show any relapse.

After 1 week, some relapse (darkening) was observed in gel plus light and gel treatment groups, but further changes up to 1 month were then minimal. Analysis of baseline shade versus the percentage change in shade revealed that darker dentin underwent greater change, particularly with gel plus light ($R = 0.494$) (Graph 1). Likewise, a similar analysis assessing percent relapse revealed that darker teeth at baseline underwent more relapse after treatment ($R = 0.207$).

DISCUSSION

The model used in the study was not intended to replicate clinical conditions but rather to serve as a proof of concept for the potential use of an intense bleaching treatment based on the photo-Fenton reaction. In the clinical situation, the overlying enamel would alter the effect on dentin achieved from an in-office bleaching treatment. Nevertheless, the system removes most of the variables which plague clinical studies (unknown type and dose of tetracycline, different pattern and severity

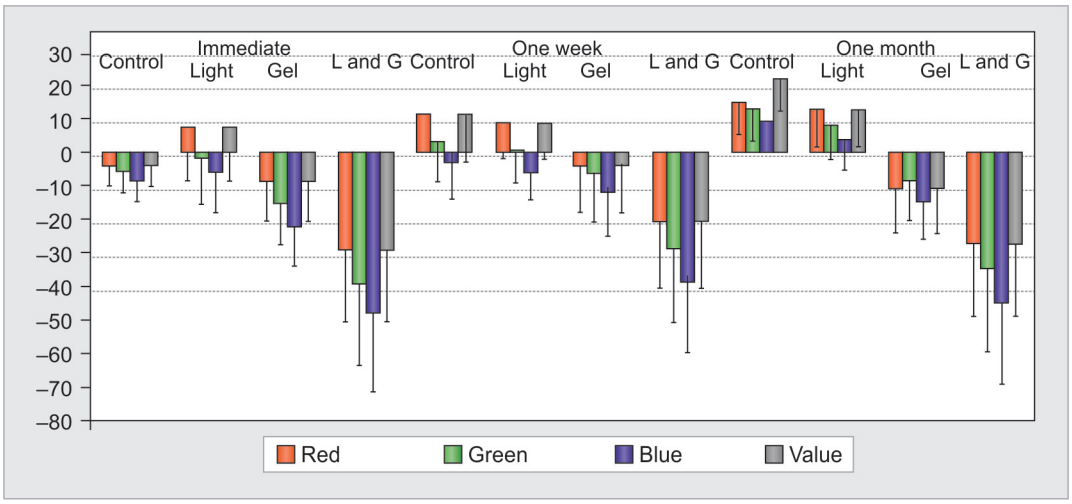


Fig. 1: Mean color change in each of the four channels at three different time periods after treatment (immediate, 1 week, 1 month), in each of the four experimental groups (control, light only, gel only, light and gel). Bars show means and SD for (N = 20)

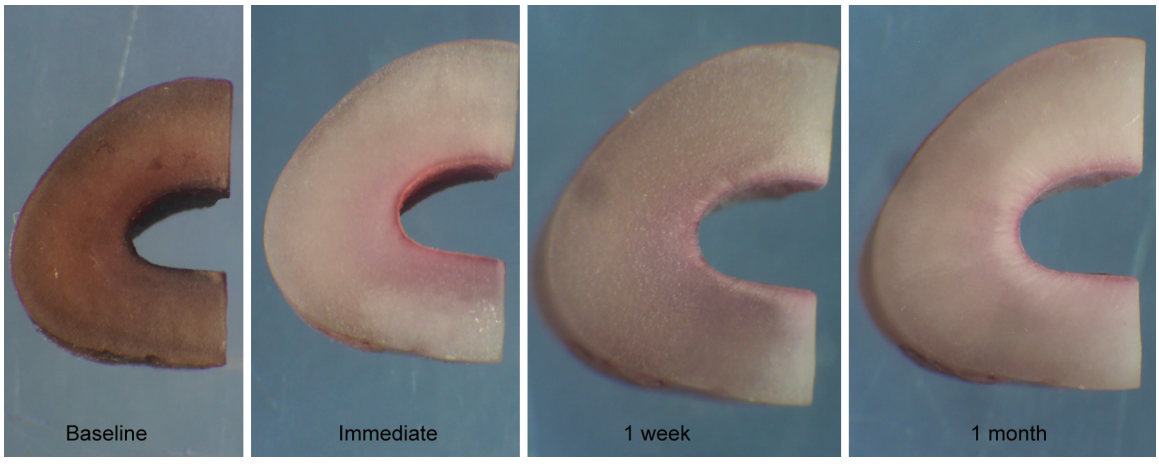
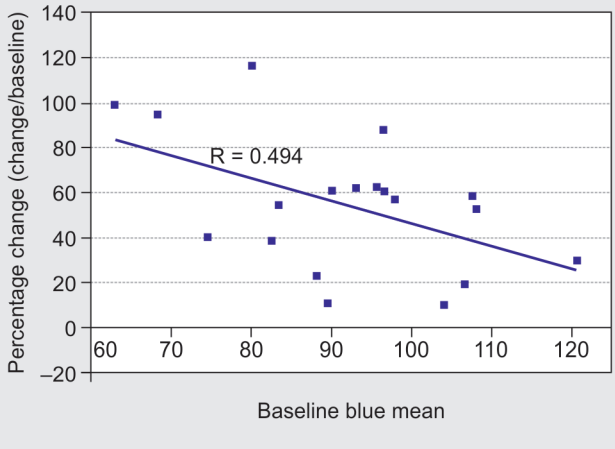


Fig. 2: Sequential images of the same root half slice treated with ZOOM! light and gel. From left to right, baseline, immediately after treatment, at 1 week and after 1 month



Graph 1: Baseline blue channel mean *vs* percentage change in the blue channel. Individual data points show each of the 20 replicates in the ZOOM! Light plus gel group. The regression line shows the correlation between the data points, illustrating the link between more yellow (less blue) at baseline and an increased amount of change

of staining, differing degrees of penetration into tooth structure for the bleaching agent, constant hydration of the teeth).

Taking on board the imperfections of the laboratory model, it nevertheless provides several useful insights into the benefits that could be achieved by the use of the photo-Fenton bleaching chemistry, namely, an overall lightening of the stained dentin, with a marked visible reduction in the intensity of yellow. There was a greater effect noted when combining the gel with the blue LED array for light activation, and no effects for the light used alone, arguing against any effect of blue light *per se* on tetracycline stained tooth structure. One would not expect a significant photo-oxidation effect of visible blue light on tetracyclines and their derivatives which form in tooth structure as the optimal wavelength for this is known to be in the visible green range.[13] The improvement from using the blue LED array to irradiate the ZOOM! gel is, however, consistent with the known chemistry of the photo-Fenton reaction.[18,19] Using 25% hydrogen peroxide gel alone without light was less effective than when light was used.

A further point of interest is that greater bleaching results were achieved with darker initial shades, which

provides some confidence that a clinical method based on using photo-Fenton bleaching to augment nightguard vital bleaching could have value. One would need to consider, however, that with greater positive change comes a greater risk of relapse. The issue of relapse has already been described for nightguard vital bleaching of tetracycline stained teeth.[17]

CONCLUSION

This proof of concept laboratory study provides evidence that tetracycline stained dentine can be lightened by the use of the Zoom! WhiteSpeed. Using the supplied blue LED array provides a greater benefit than using gel alone. Further work is needed to assess the value of such systems when used on intact teeth in both laboratory and clinical settings. In the future, it may be worthwhile to combine such in-office treatments with nightguard tray-based bleaching to reduce the overall length of treatment and lessen the need for full coverage restorations.

CLINICAL SIGNIFICANCE

Results of this proof of concept laboratory study suggest that a light-activated bleaching system utilizing the photo-Fenton reaction can help to lighten tetracycline-stained dentin. Thus, bleaching treatments based on the photo-Fenton reaction, such as Zoom! WhiteSpeed, may have benefits as adjuncts to home bleaching for patients with tetracycline-staining. Further investigation of the use of this method for treating tetracycline-stained teeth as an adjunct to the use of tray-based bleaching in cases of tetracycline-staining would be worthwhile.

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REFERENCES

1. Davies AK, Cundall RB, Dandiker Y, Slifkin MA. Photo-oxidation of tetracycline adsorbed on hydroxyapatite in relation to the light-induced staining of teeth. J Dent Res 1985;64(6):936-939.
2. Sanchez AR, Rogers RS, 3rd, Sheridan PJ. Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. Int J Dermatol 2004;43(10):709-715.
3. Schuster A, Shwachman H. The tetracyclines; applied pharmacology. Pediatr Clin North Am; 1956. p. 295-303.
4. Kim ST, Abbott PV, McGinley P. The effects of Ledermix paste on discoloration of immature teeth. Int Endod J 2000; 33(3):233-237.
5. Kim ST, Abbott PV, McGinley P. The effects of Ledermix paste on discoloration of mature teeth. Int Endod J 2000;33(3): 227-232.
6. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod 2010;36(6):1086-1091.
7. Chen BK, George R, Walsh LJ. Root discoloration following short-term application of steroid medicaments containing clindamycin, doxycycline or demeclocycline. Aust Endod J 2012;38(3):124-128.
8. Thomson AD, Athanassiadis B, Kahler B, Walsh L. Tooth discoloration: staining effects of various sealers and medicaments. Aust Endod J 2012;38(1):2-9.
9. Ahmed HM, Abbott PV. Discoloration potential of endodontic procedures and materials: a review. Int Endod J 2012;45(10):883-897.
10. Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: a literature review. Dent Traumatol 2013;29(1):2-7.
11. Abou-Rass M. The elimination of tetracycline discoloration by intentional endodontics and internal bleaching. J Endod 1982;8(3):101-106.
12. Lin LC, Pitts DL, Burgess LW Jr. An investigation into the feasibility of photobleaching tetracycline-stained teeth. J Endod 1988;14(6):293-299.
13. Kuzekanani M, Walsh LJ. Quantitative analysis of KTP laser photodynamic bleaching of tetracycline-discolored teeth. Photomed Laser Surg 2009;27(3):521-525.
14. Matis BA, Wang Y, Eckert GJ, Cochran MA, Jiang T. Extended bleaching of tetracycline-stained teeth: a 5-year study. Oper Dent 2006;31(6):643-651.
15. Haywood VB, Heymann HO. Nightguard vital bleaching. Quintessence Int 1989;20(3):173-176.
16. Haywood VB, Leonard RH, Dickinson GL. Efficacy of six months of nightguard vital bleaching of tetracycline-stained teeth. J Esthet Dent 1997;9(1):13-19.
17. Leonard RH Jr, Van Haywood B, Caplan DJ, Tart ND. Nightguard vital bleaching of tetracycline-stained teeth: 90 months post treatment. J Esthet Restor Dent 2003;15(3):142-152.
18. Lagori G, Rocca J, Brulat N, Merigo E, Vescovi P, Fornaini C. Comparison of two different laser wavelengths' dental bleaching results by photo-Fenton reaction: in vitro study. Lasers Med Sci 2015;30(3):1001-1006.
19. Ziemba SL, Felix H, MacDonald J, Ward M. Clinical evaluation of a novel dental whitening lamp and light-catalyzed peroxide gel. J Clin Dent 2005;16(4):123-127.