WHITENING EFFECT OF BLEACHING AGENTS ON TETRACYCLINE-STAINED RAT TEETH

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ABSTRACT

테트라싸이클린에 의해 변색된 백서 치아에 대한 표백제의 표백 효과

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본 연구는 테트라싸이클린에 의해 변색된 백서 치아에 대한 3종 표백제의 표백효과를 비교하고 표백제의 침투 깊이(법랑질 두께)에 따른 표백효과의 차이를 판별하기 위해 시행되었다. 30 마리의 웅성 백서에 매일 2주 동안 테트라싸이클린 용액을 복강내 주입하였다.

디스크 형태의 32개 백서 치아 표본을 16시간 동안 자외선으로 조사하여 변색을 야기시켰다. 대조군은 생리 식염수에 보관하였으며 실험군은 3종 표백제(Opalescence, Rembrandt, NiteWhite)에 하루 5회씩 2주간 노출시켰다. 치아 색상을 표백전, 표백 1일, 3일, 5일, 7일, 9일, 11일, 및 14일에 각기 기록하여 치아내 변색부와 정상 색상간의 색상차(△E)를 판독하였다: 대조군 - 9.78, 9.17, 9.36, 9.65, 9.40, 9.99, 10.57, 11.36; Opalescence - 10.08, 7.63, 6.72, 6.04, 5.10, 4.87, 4.89, 4.27; Rembrandt - 9.83, 11.27, 9.55, 8.36, 7.75, 6.94, 7.11, 7.04; NiteWhite - 10.44, 9.92, 7.58, 6.80, 5.45, 5.05, 4.73, 4.01. 표백된 모든 치아들의 색상이 밝아졌다. ($p<.01$)

테트라싸이클린이 주입된 56개 백서 치아를 3일간 자외선에 조사한 다음, 3가지 유형의 표백제 침투깊이를 설정하였다: 설측 상아질과 순측 법랑질을 통한 침투 (DN), 순측 법랑질만을 통한 침투 (RE), 1.0 mm 두께의 사람 법랑질로 피개된 순측 법랑질을 통한 침투 (HE). 표본들을 Opalescence로 하루 5회씩 1주일 및 4주간 표백한 후 횡단하여 치아 색상을 측정하였다. 표백되지 않은 치아들을 대조군으로 이용하였다. 치아내 변색부와 정상 색상간의 색상차(△E)는 다음과 같았다: 대조군 - 11.67; 1주 DN군 - 13.55; 1주 RE군 - 12.80; 1주 HE군 - 12.07; 4주 DN군 - 7.48; 4주 RE군 - 7.50; 4주 HE군 - 11.69. 4주 DN군과 4주 RE군의 색상 변화가 가장 크게 나타났다. ($p<.01$)

주요어: 표백, 침투깊이, 테트라싸이클린, 색상차, 색상 변화

I. INTRODUCTION

Tooth shade, which is the result of diffuse reflectance from the inner dentin through the outer translucent enamel layer[1], is believed to be the most important factor in patients' perceptions of dental attractiveness[2]. Because tooth discoloration contributes to psychological distress as well as poor esthetics, various techniques have long been sought to regain natural tooth color. The use of veneers or esthetic crowns to cover unesthetic teeth is one way to furfill this purpose. However, bleaching techniques are preferred because tooth color can be regained without removing sound, healthy tooth structure.

Bleaching has become a routine, popular, conservative option with a high rate of success. Unfortunately, some regression does occur and not all tooth discoloration can be corrected. One such case is discoloration due to tetracycline(TC), which is very difficult to bleach. TC discoloration occurs when TC has been

ingested at a young age or by the patient's mother during pregnancy. Mello found that TC chelates with calcium at the hydroxylapatite surface of mineralizing dentin to form tetracycline-orthophosphate which results in tooth discoloration[3]. It was also noted that TC deposited in enamel or dentin is permanently positioned while that absorbed in bone could be released through normal bone remodeling[4]. This might be one reason why this stain responds less to bleaching. The discoloration is seen predominantly in dentin and cementum, and only slightly in enamel, because of dentin's greater absorbance of TC due to a larger surface area of dentin apatite crystals than that of enamel apatite crystals[5].

Several methods, including vital bleaching and intentional nonvital bleaching[6], have been used to lighten the color of TC-stained teeth. But the extent of lightening was not comparable to that of non-TC-stained teeth with vital bleaching[7]. In one study in which teeth were devitalized, and a non-vital bleaching technique used, long-term results were not favorable[8]. Furthermore, non-vital bleaching has been associated with a risk of external root resorption[8].

It is certainly better to perform a vital bleaching technique for TC-stained teeth rather than to devitalize the teeth and use a non-vital bleaching technique, but vital bleaching agents currently in use, have limited efficacy in severe TC stain cases. Haywood, however, reported recently that extending vital bleaching time to 6 months would allow moderate to severe TC discoloration cases to respond to treatment, and that the best indicator of a good prognosis is not the severity of the discoloration, but the location of the discoloration[9]. The reasons for the decreased response of TC-stained teeth may be that the bleaching potential of these agents on TC stain may be ineffective or that they may fail to penetrate deep enough through the enamel into the dentin.

The objectives of this work were to compare the whitening effects of 3 bleaching agents and to elucidate the differences in bleaching effect when the depth of penetration of the bleaching agents was varied. The amount of color change was quantitated by computer-aided image processing instead of subjectively comparing before- and after-treatment photographs[10,11].

II. MATERIALS AND METHODS

Thirty Albino rats(wt. 300-350 gm) were peritoneally injected daily for 2 weeks with 6 ml of 5 mg/ml TC solution[Chlortetracycline(Teracyclin$_{cap}$, ChongKeunDang, Korea)]. One week after the final injection, the rats were sacrificed by cervical dislocation, and the teeth were extracted and coded with random two-digit numbers. Thirty-two mandibular teeth were used in the first phase of this study which compared the effectiveness of 3 vital bleaching agents. Fifty-six maxillary teeth were used in the second phase to identify the differences in bleaching effect when the depth of penetration of the bleaching agent was varied.

1) Specimen preparation

Teeth were sectioned to separate the coronal and root portions. Pulpal remnants were carefully removed with a barbed broach. The teeth were cleaned in an ultrasonic bath and were stored in saline solution in a light-proof case to prevent color change due to light exposure. For the first phase, the root and the cervical 1 mm of the stained crown were ground away, and a 1.5 mm thick disc was made by horizontal sectioning with a carborundum disc. Discs were polished with 600 grit sandpaper to prevent any effects from irregular texture[12].

The teeth were exposed to ultraviolet(UV) light since it has been reported that TC-induced tooth color shifts into the yellow-green hue[13] and then gradually darkens to brown or gray-brown with continued UV light exposure due to the formation of a red quinone product[14]. To induce maximum discoloration, the thirty-two sections were irradiated for 16 hours with UV light(H-5000A, Hanshin medical co., Korea) and the fifty-six intact maxillary TC-stained teeth were irradiated for 72 hours. These irradiation times for the two types of specimens were determined as the times necessary to assure significant brown discoloration.

2) Bleaching procedures

i) Phase 1 - effectiveness of 3 bleaching agents

The thirty-two discs were randomly divided into 4 groups of 8 discs each, a control group and three bleaching groups. The bleaches used were 10% carbamide peroxide bleaching agents of three brands: Opalescence(Ultradent), Rembrandt(DenMat), and NiteWhite(Discus dental). Bleaching agents were applied to samples for 1 hour, five times a day for 14 days. Teeth sections in the control group were stored in saline solution. After the each 1-hour immersion in the bleaching agent, each specimen was rinsed with tap water. After the fifth 1-hour bleaching, the specimens were stored in saline solution until the next day. All bleaching was carried out in a light-proof case.

Images of the teeth were taken to check the color space (L^*, a^*, b^*) at the following times: prior to initiating bleaching, day 1, day 3, day 5, day 7, day 9, day 11, and day 14.

ii) Phase 2 - effect of overlying enamel thickness on bleaching

To determine the effect of penetration depth on bleaching, three different penetration depths were tested: penetration through lingual dentin and labial enamel(DN), penetration through labial enamel only (RE), and penetration through 1.0 mm thickness of human enamel attached to labial rat enamel(HE). This also allowed the determination of whether increased bleaching is possible when enamel is thinned or the outer dentin is exposed by grooving in severely stained teeth.

Because rat teeth don't have lingual enamel, teeth in the DN group were exposed without alteration to the bleaching agent. This group was used to simulate human teeth with dentin exposed via grooving the enamel which calls for the least penetration depth. In the RE group, the exposed lingual dentin was covered with 2 coats of nail polish and wax so that the bleaching agent would penetrate into tooth structure through the labial enamel only. It was noted that rat labial enamel is composed of an outermost amorphous, aprismatic layer and inner prismatic layer similar to human enamel, but thinner[13]. This group was used to simulate human teeth in which some part of the labial enamel was thinned by grooving the enamel without dentin exposure. In the HE group, the bleaching agents had to penetrate into the rat teeth through a 1.0 mm thickness of human enamel, which was attached to the labial surfaces of the rat teeth using paper tape. This group was used to simulate intact human teeth with normal enamel, which calls for the largest penetration depth.

The fifty-six maxillary intact teeth, which had been UV irradiated for 3 days, were randomly divided into 7 groups. For the control group, eight teeth were sectioned(1.5 mm thick disc) without bleaching and their color spaces were examined. The color spaces from control teeth were used as baseline values. For the experimental groups, Opalescence was chosen as a bleaching agent because of its strong bleaching potential. Three groups of intact teeth, prepared as described above, were bleached 5 times a day for 1 week. In another 3 groups, this bleaching procedure was continued for 4 weeks. After sectioning and obtaining color spaces from bleached teeth, bleached teeth were compared to the teeth in the control group.

3) Analysis of the color spaces

Tooth color was analyzed with the CIELAB system. This L^*, a^*, b^* color is related to human color perception in all three dimensions or directions of color space. L^* represents the degree of gray and corresponds to value or brightness; a^* is a hue-chroma parameter in the red-green direction; b^* is a hue-chroma parameter in the blue-yellow axis. Equal distances in the color space were approximately equally perceived.

Images were captured via a stereomicroscope(15x), using a CCD camera(1k-642k, Toshiba, Japan). The computer software, HumanEye(Professional Scientific Instrument Co., Korea) was used for assessing the tooth color. The change of color difference($\triangle E$) between the stained area and non-stained peripheral dentin area was measured rather than the change of color within the stained area itself. Color spaces at 8 different stained and 8 different non-stained areas were measured and their mean values were estimated. Color difference($\triangle E$) between these two values was calculated by the equation: $\triangle E=[(\triangle L^*)^2+(\triangle a^*)^2+(\triangle b^*)^2]^{1/2}$

4) Statistical analysis

For the first phase of this study, differences between color shifts of all groups were compared using a repeated-measures analysis of variance (MANOVA) and with a Newman-Keuls Post Hoc Test. One-way Analysis of Variance(ANOVA) and a Least-significant difference Post Hoc Test were used for the second phase of this study to detect any differences among groups.

III. RESULTS

1) Phase 1

Table 1 shows the color differences of △E values between stained areas and non-stained peripheral dentin areas of all groups. All bleached teeth were lightened in general, and △E values were reduced. The teeth in the control group showed relatively stable values. There was no difference between groups at the beginning of this experiment:control [9.78(4.18)], Opalescence[10.08(3.11)], Rembrandt [9.83(3.70)], NiteWhite[10.44(1.92)](Fig. 1). Although the rate with which teeth lightened decreased with time, there was no significant difference between groups until day 5(Fig. 2). At day 5, bleaching agents of Opalescence and NiteWhite showed significantly greater bleaching than the control and Rembrandt groups(Fig. 3). Of all 3 bleaching agents, Rembrandt could not effectively bleach TC-stained teeth until day 9(Fig. 4, 5) (Table 2). Table 3 shows that all 3 bleaching agents effectively bleached TC-stained teeth, but Opalescence and NiteWhite were significantly more effective than Rembrandt.

Table 1. △E values between stained areas and non-stained areas of all groups Mean(S.D.)

	Control	Opalescence	Rembrandt	NiteWhite
Before bleaching	9.78 (4.18)	10.08 (3.11)	9.83 (3.70)	10.44 (1.92)
Day 1	9.17 (3.93)	7.62 (2.51)	11.27 (3.42)	9.92 (1.84)
Day 3	9.36 (4.14)	6.72 (1.74)	9.55 (2.77)	7.58 (1.64)
Day 5	9.65 (4.23)	6.04 (1.50)	8.36 (2.16)	6.80 (0.89)
Day 7	9.40 (3.74)	5.10 (1.95)	7.75 (1.63)	5.45 (1.08)
Day 9	9.99 (5.05)	4.87 (2.17)	6.84 (2.05)	5.05 (1.25)
Day 11	10.57 (5.33)	4.89 (2.27)	7.11 (2.10)	4.73 (1.40)
Day 14	11.36 (4.82)	4.27 (2.25)	7.04 (2.35)	4.01 (1.02)

Table 2. Daily change of p values among groups

	p value	Remarks
Before bleaching	.977	
Day 1	.136	
Day 3	.139	
Day 5	.036	Control, Remrandt < NiteWhite, Opalescence
Day 7	.003	Control, Remrandt < NiteWhite, Opalescence
Day 9	.007	Control < Remrandt, NiteWhite, Opalescence
Day 11	.003	Control < Remrandt, NiteWhite, Opalescence
Day 14	.000	Control < Remrandt, NiteWhite, Opalescence

Table 3. MANOVA result among groups (Newman-Keuls test)

	Control	Opalescence	Rembrandt	NiteWhite
Control				
Opalescence	.000008*			
Rembrandt	.005048*	.000049*		
NiteWhite	.000022*	.284622	.000810*	

* p<.01

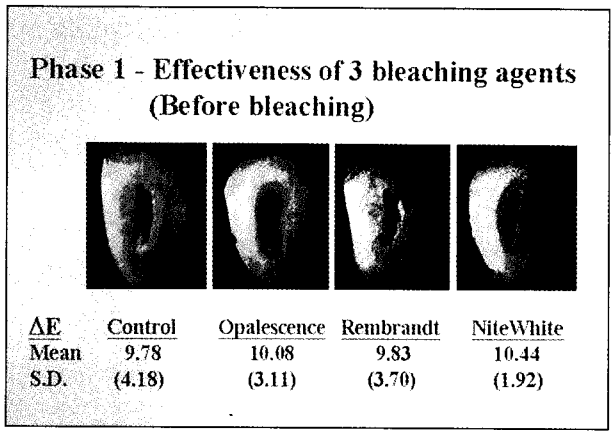

Fig. 1. Representative images in phase 1 study; before bleaching

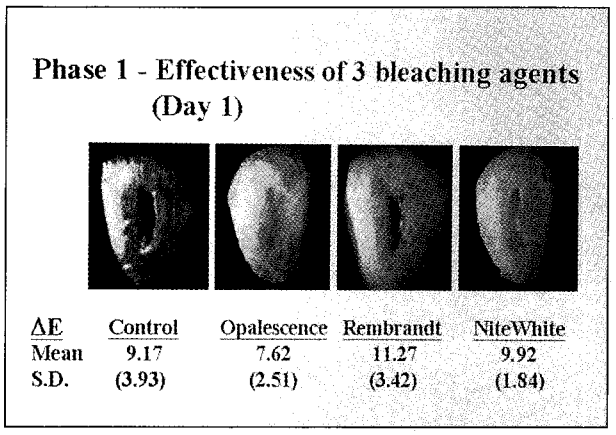

Fig. 2. Representative images in phase 1 study; day 1

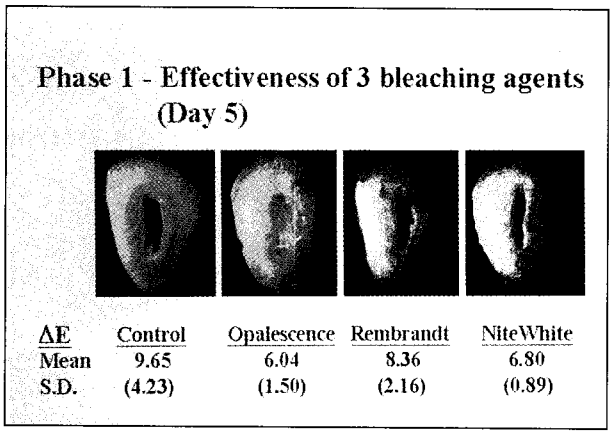

Fig. 3. Representative images in phase 1 study; day 5

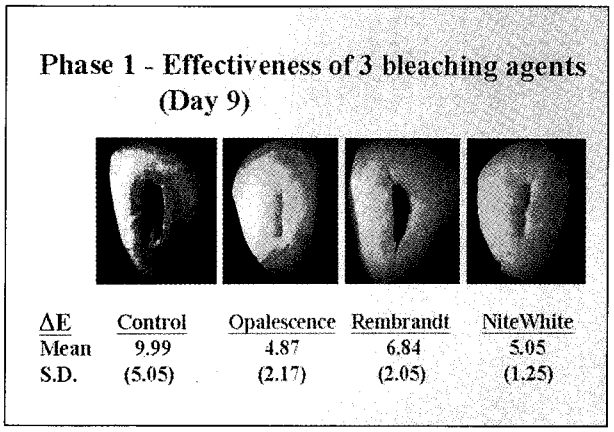

Fig. 4. Representative images in phase 1 study; day 9

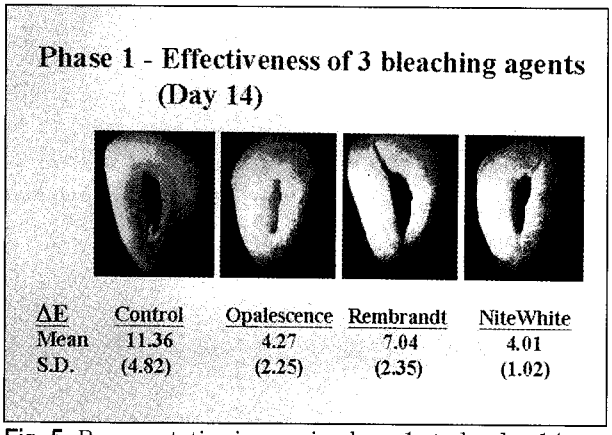

Fig. 5. Representative images in phase 1 study; day 14

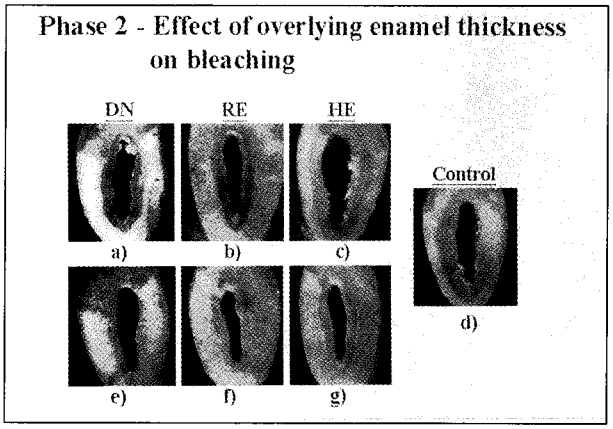

Fig. 6. Representative images in phase 2 study
a) 1 week DN group b) 1 week RE group
c) 1 week HE group d) control group e) 4-week DN group f) 4-week RE group g) 4-week HE group

It was not the objective of this study to evaluate any damage to teeth, such as crack formation or fracture, but cracks could be seen in some of the specimens in all groups, including the control group. There was surprisingly less crack formation in the Opalescence group(2/8) than in the control group

Table 4. Number of specimens of crack and fracture

	No. of specimens	Normal	Crack	Fracture
Control	8	2	6	0
Opalescence	8	6	2	0
Rembrandt	8	0	1	7
NiteWhite	8	2	4	2

Table 5. △E values of all groups in phase 2 study

Groups	Bleaching time	△E	
		Mean	S.D.
Control	0	11.68	3.29
DN	1 week	13.55	1.87
	4 weeks	7.48*	2.23
RE	1 week	12.80	3.52
	4 weeks	7.50*	1.96
HE	1 week	12.07	2.27
	4 weeks	11.69	2.74

* $p<.05$

(6/8). One-fourth of the specimens in the NiteWhite group and nearly all specimens in Rembrandt group were fractured, while the control and Opalescence groups showed no fractures(Table 4).

2) Phase 2

Fig. 6 shows representative images. Means and standard deviations of △E values between stained areas and non-stained peripheral dentin areas in all 7 groups are given in Table 5. After 1 week, there was no difference between groups. After 4 weeks of bleaching with Opalescence, the greater color changes were found in the groups of exposed dentin(DN) and thinned enamel(RE). Compared with the groups bleached for 1 week, the 4-week whitening effect was significantly better($p<.01$) in all groups except the HE group(Table 6).

IV. DISCUSSION

Bleached groups showed decreasing △E values with time. Teeth were lightened($p<.05$) and their chromas (degree of stain) were reduced. Lenhard reported that the observed tooth color change after bleaching was mainly due to a change in the color parameters L^* and b^*, and that bleaching causes a shift in the blue direction within the color space and lightens the color of the teeth[15]. Although these factors were not evaluated statistically because they were outside of our objectives in this study, the same tendency could also be observed: increased L^*, decreased a^*(shift toward the green direction), and decreased b^* spaces (shift toward the blue direction).

Table 6. Statistical differences between 1-week and 4-week groups

		Mean difference	p value
△E	DN 1 week vs 4 weeks	6.0725	.000
	RE 1 week vs 4 weeks	5.2950	.000
	HE 1 week vs 4 weeks	.3800	.773

As compared with the control group, significantly bleached teeth were seen in all 3 bleaching groups. There was no significant difference between the Opalescence and NiteWhite groups, which both showed better bleaching than the Rembrandt group. Opalescence and NiteWhite showed differential bleaching effects from day 5, while Rembrandt showed effects from day 9(Table 3). The bleaching effect of Rembrandt was slower and less effective. Among the 3 bleaching agents, Opalescence and NiteWhite showed a similar changing pattern of the △E value, but the day of the greatest change was different in each of those groups: day 1 was greatest for Opalescence and day 3 for NiteWhite. This showed that Opalescence was a faster-acting agent.

The aim of dental bleaching is to remove stains so that ultimately one could not discern the difference between color spaces of bleached areas and normal areas. There are varying opinions as to the amount of color difference needed to differentiate between the two colors. Kuehni and Marcus reported that color differences greater than 1.0 are perceivable by more than 50% of observers under uniformly controlled conditions[16]. Johnston and Kao, who compared a visual rating scale with colorimetry of composite restorations and natural teeth, found that the average CIELAB color difference of tooth/restoration and restoration/restoration pairs judged visually to be an acceptable match was 3.7[12]. After 2 weeks of bleaching in this study, Opalescence and NiteWhite achieved an △E value approximately 4.5. By either the 1.0 or 3.7 criterion, this color difference is detectable. It is difficult, however, to discern between any normal peripheral dentin area and the stained

area after bleaching. Further study is needed to determine how this difference of 4.5 is perceived.

Haywood et al. reported that there was no obvious difference between the texture of bleached teeth and unbleached teeth as seen with scanning electron microscopy[10]. Bleaching is believed to be safe if it is controlled and monitored by a dentist. As indicated in a Table 4, cracks could be seen in some of the specimens in all groups including the control group. There were surprisingly fewer cracks seen in the Opalescence group(2/8) than in the control group (6/8). One-fourth of the specimens in the NiteWhite group and nearly all specimens in Rembrandt group were fractured, while control and Opalescence groups showed no fracture. It is possible that Opalescence has an ingredient that increases tooth resistance to dehydration and cracking or fracturing. Longer application might bring about increased cracking and fracturing. In this study, bleaching agents were applied to samples 5 times a day for 2 weeks for a total of 70 hours of bleaching. If a patient were to wear a bleach carrier for 2 hours a day, it would take 35 days or 5 weeks to achieve 70 hours of bleaching.

From the second phase of this study, the effect of the thickness of enamel on bleaching was observed. Teeth bleached for 1 week didn't show any significant color change from the control group. This result was different from that of the first phase of this study. Even a little thickness of permeable dentin retarded the rate of penetration of the bleaching agent. Compared with the results of groups bleached for 1 week, significant color changes were found in the 4-week groups of exposed dentin(DN) and thinned enamel(RE)($p<.01$), but there was no difference in the HE group(covered with 1.0 mm thick human enamel). The thick enamel acts as an effective barrier to the vital bleaching agent, Opalescence. It could not penetrate sufficiently to bring about bleaching of deeply stained enamel or dentin. Thus, when teeth are so severely stained that routine bleaching procedures have no effect when the agent is applied to the surface, it may be possible to whiten those teeth if the deeper enamel is exposed directly to the bleaching agent through a groove cut into the enamel without exposing dentin.

Conventional, photographic methods were used to detect bleaching effect until the development of instruments such as a colorimeter[15,17-19]. This instrument is known to convert all colors within the range of human perception into a common numerical code allowing a more quantitative comparison, and is now being used more frequently. In this study, the image of rat teeth was captured through a stereomicroscope and CCD camera, because the rat teeth are too small to use the colorimeter. But this method has drawbacks, namely, the difficulty in achieving uniform illumination from image to image, affecting the color of the image.

V. CONCLUSION

All the bleaching agents demonstrated enough power to bleach TC stains, but it is the ability of these agents to penetrate the tooth structure that determines clinical efficacy. Manufacturers should concentrate efforts to develop ways to increase the penetration potential of bleaches. Also, in severely TC-stained cases that are not responsive to routine vital bleaching, thinning or grooving the overlying enamel may allow penetration of bleaching agents into stained dentin.

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REFERENCES

1. O.Brien WJ: Double layer effect and other optical phenomena related to esthetics. DCNA 29(4):667-672, 1985
2. Dunn WJ, Murchison DF, Broome JC: Esthetics: patient's perceptions of dental attractiveness. J of Prosth 5(3):166-171, 1996
3. Mello HS: The mechanism of tetracycline staining in primary and permanent teeth. J Dent Child 34:478-482, 1967
4. Stewart DJ: The re-incorporation in calcified tissues of TC released following its deposition in the bone of rats. Arch Oral Biol 18:759-764, 1973
5. Urist M, Ibsen K: Chemical reactivity of mineralized tissue with oxytetracycline. Arch Pathol 76:484-496, 1963
6. Aldecoa EA, Mayordomo FG: Modified internal bleaching of severe tetracycline discoloration: a 6-year clinical evaluation. Quint Int 23(2):83-89, 1992
7. Haywood VB, Heymann HO: Nightguard vital bleaching.

Quint Int 20;173-176, 1989
8. Friedman S: Internal bleaching: long-term outcomes and complications. JADA 128(Suppl);51S-55S, 1997
9. Haywood VB: Nightguard vital bleaching: current concepts and research. JADA 128(Suppl);19S-25S, 1997
10. Haywood VB, Leonard RH, Dickinson GL: Efficacy of six months of nightguard vital bleaching of tetracycline-stained teeth. J Esth Dent 9(1);1-7, 1996
11. Haywood VB, Leonard Jr RH, Nelson CF: Efficacy of foam liner in 10% carbamide peroxide bleaching technique. Quint Int 24(9);663-666, 1993
12. Johnston WM, Kao EC: Assessment of appearance match by visual observation and clinical colorimetry. J Dent Res 68;819-822, 1989
13. Shin DH, Cho YB: Tooth color and structure changes induced by tetracycline in rat. J of Korean Academy Conservative Dentistry 21(2);664-676, 1996
14. Walton RE, O'Dell NL, Myers DL, Lake FT, Shimp RG: External bleaching of tetracycline stained teeth in dogs. JOE 8(12);536-542, 1982
15. Lenhard M: Assessing tooth color change after repeated bleaching in vitro with a 10 percent carbamide peroxide gel. JADA 127(11);1618-1624, 1996
16. Kuehni FG, Marcus RT: An experiment in visual scaling of small color differences. Color Res Appl 4;83-91, 1979
17. Rustogi KN, Curtis J: Development of a quantitative measurement to assess the whitening effects of two different oxygenating agents on teeth in vivo. Compendium 17(Suppl);631S-634S, 1994
18. Kowitz GM, Nathoo SA, Wong R: Clinical comparison of Colgate Platinum Tooth Whitening System and Rembrandt Gel Plus. Compendium 17(Suppl);646S-651S, 1994
19. Nakamura T, Nakajima H, Salimee P, Hino T, Maruyama T: Effect of bleaching on vital discoloured teeth-a colorimetric evaluation in three patients. Asian J of Asthet dent 1(1);25-28, 1993