Tooth discolouration and internal bleaching after the use of triple antibiotic paste

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Abstract

Aim To assess the discolouration of teeth with closed and open apices after placement of triple antibiotic paste (TAP, ciprofloxacin, metronidazole and minocycline) in the pulp chamber and whether discolouration could be reversed by internal bleaching procedures.

Methodology Twenty extracted human mandibular premolars were divided into 2 groups (n = 10): teeth with closed apices (CA) and teeth with open apices (OA). After conventional access, the TAP was sealed in the pulp chamber for 3 weeks. The paste was removed by a rinse with sodium hypochlorite (NaOCl) and a mixture of sodium perborate and distilled water was sealed in the pulp chamber for 1, 2 and 3 weeks. The shade was measured by a spectrophotometer at six time periods: baseline (T0), after 3 weeks of placement of TAP (T1), after removal of TAP with a NaOCl rinse (T2) and after 1 (T3), 2 (T4) and 3 (T5) weeks of internal bleaching with sodium perborate paste. Data were collected based on the CI-ELAB-CIE1976 (L*a*b*) system and analysed using t-tests and ANOVA.

Results A significant decrease in the mean values of L* (lightness) was observed after treatment with TAP (T1, P < 0.05). Considerable increases in these values after bleaching with sodium perborate (T3 < T4 < T5) were found in both groups. The only significant difference in the intergroup analysis was between T1 and T2, in which ΔE values in the OA group were higher (P = 0.04).

Conclusions TAP discoloured the tooth structure, but discolouration could be reversed with sodium perborate. In general, teeth with closed and open apices had the same rates of discolouration and bleaching.

Keywords: antibiotics, bleaching, discolouration, sodium perborate.

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Introduction
Traditionally, the treatment of a tooth with an open apex and a necrotic pulp was achieved with apexification procedures either with long-term use of calcium hydroxide or by placement of mineral trioxide aggregate as an apical plug. Apexification permits disinfection and filling of the root canal space with no further development of the root (Raldi et al. 2009). Revascularization is a regenerative endodontic procedure and a contemporary option for the same type of cases. Restoration of the blood supply will take place, inducing continuing root development by deposition of hard tissue and resulting in thickening of the canal walls, lengthening of the root, and apical closure (Banchs & Trope 2004, Chueh & Huang 2006, Jung et al. 2008, Chueh et al. 2009, Ding et al. 2009, Petrino et al. 2010).
Intracanal medicaments used in revascularization procedures vary and include calcium hydroxide, formocresol and pastes that combine antibiotics (Moreno-Hidalgo et al. 2014). A triple antibiotic paste (TAP, mixture of ciprofloxacin, metronidazole and minocycline) has been proposed as one intracanal medicament to disinfect the root canal system for revascularization procedures (Hoshino et al. 1996, Iwaya et al. 2001, Banchs & Trope 2004, Jung et al. 2008, Ding et al. 2009, Petrino et al. 2010). TAP is formed by mixing equal parts of all three antibiotics in powder form with saline or sterile water. This paste is sealed in the canal for 3 weeks. One side effect of the use of the TAP is the discolouration of the tooth structure and the crown in particular resulting in an unaesthetic appearance (Kim et al. 2010, Dabbagh et al. 2012).

Discolouration of a root filled tooth can be improved by placing oxidizing agents in the pulp chamber. In the walking bleach technique, an oxidizing agent is placed in the pulp chamber and sealed for a period of time whilst whitening of the tooth is assessed. A common bleaching agent is sodium perborate, a powder that when in contact with air or moisture gradually releases low levels of hydrogen peroxide, a main bleaching agent (Abbott & Heah 2009).

Revascularization has emerged as a favourable treatment alternative, in particular for teeth that underwent pulp necrosis in the early stages of root formation. This treatment may result in root lengthening and increased dentine width, possibly decreasing the risk of tooth fracture (Thibodeau & Trope 2007, Shin et al. 2009). Although other medicaments have been proposed for revascularization procedures, TAP seems to be used commonly (Diogenes et al. 2013, Moreno-Hidalgo et al. 2014). The potential conflict is that, although it should stay within the root canal, TAP may come into contact with the pulp chamber and discolour the crown of the tooth, which will pose an aesthetic problem. Excellent results can be achieved with internal bleaching to correct intrinsic tooth discolouration. The results may vary depending on the type of discolouration and length of time that the tooth has been discoloured. To date, there is no research to verify whether the discolouration of teeth treated with TAP can be improved by bleaching procedures.

The aims of this study were to (i) confirm whether tooth discolouration occurs when TAP is used as a medicament; (ii) verify whether discolouration caused by TAP can be reversed by bleaching procedures and (iii) assess whether there is a difference in the pattern of discolouration and bleaching between teeth with open and closed apices.

**Materials and methods**

This study was approved by the Research Ethics Board of the University of Manitoba (H2014:106).

**Sample selection**

Twenty extracted human mandibular premolars (10 with closed apices and 10 with open apices) were used. All teeth were cleansed and then radiographed to verify the absence of resorption or previous endodontic treatment. Teeth identified with an open apex had their apical foramen measured with a digital caliper, and this diameter had to be >1.5 and <2.5 mm. The teeth were sterilized in an autoclave and were immersed in saline for hydration in individually labelled bottles for 72 h.

**Sample preparation**

The access opening was achieved, and all contents of the pulp chamber and coronal portion of the root canal were removed with a size 15 hand file (Dentsply Tulsa Dental, Tulsa, OK, USA) and distilled water. A cotton pellet was placed in the pulp chamber, and the access preparation was sealed with Cavit (3M Espe, MN, USA).

**Shade recording**

The samples were positioned in a plaster apparatus, and a silicone impression was made over the teeth. Perforations were made in the centre of the buccal surface of the silicone impression to standardize the procedure for shade recording. Shade recording of the crown was performed with a Vita Easyshade Advance 4.0 spectrophotometer (Vita Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany). This equipment quantifies the shade through a system named CIELAB-CIE 1976 (L*a*b*) (Commission Internationale de L’Eclairage, Vienna, Austria) (Westland 2003). The L* value indicates lightness and varies between 0 for black and 100 for white; a* determines the amount of red (positive values) or green (negative values); and b* exhibits the amount of yellow (positive values) or blue (negative values).
The shade was measured in the same room under the same light by one calibrated operator at six different time periods: baseline (T0), after 3 weeks of placement of TAP (T1), after rinsing out TAP with 2.5% sodium hypochlorite (NaOCl) (T2), 1 week after placement of sodium perborate in the pulp chamber (T3), 2 weeks after placement of sodium perborate in the pulp chamber (T4) and 3 weeks after placement of sodium perborate in the pulp chamber (T5). At each time period, three measurements of L*, a* and b* were recorded and ΔL, Δa and Δb were then calculated by subtracting the final data from the initial data within each time period. The constant difference in colour (ΔE) was then calculated using the following formula: \( \Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \) (FriedelMax Adam-Chickering - Comission Internacionale de L’Eclairage, 1976).

**Experimental procedures**

The specimens were assigned to an experimental group (n = 10) according to the dimension of the apex: CA – teeth with closed apices and, OA – teeth with open apices.

A baseline shade was taken before any material was placed in the pulp chamber (T0). The Cavit (3M Espe) and the cotton pellet were removed, and the TAP was mixed with distilled water to a thick consistency. The mixture was placed in the pulp chamber with a plastic instrument, and the access cavity was again sealed with Cavit (3M Espe). Three weeks later, the tooth shade was recorded (T1). The Cavit (3M Espe) was removed, and the medicament was rinsed out of the tooth with 5 mL of 2.5% NaOCl. The pulp chamber was inspected to ensure that the medicament had been completely removed, and the shade was again recorded (T2). For the bleaching procedure, sodium perborate powder (Sultan, Hackensack, NJ, USA) was mixed with distilled water (2 g:1 mL ratio) to a thick consistency, placed in the pulp chamber with a plastic instrument and then sealed with Cavit (3M Espe) for 1 week. After 1 week, the shade was again taken (T3 = 1 week of bleaching). After removing the temporary filling, the pulp chamber was thoroughly rinsed with 5 mL of 2.5% NaOCl and a fresh sodium perborate paste placed in the pulp chamber and sealed for 1 additional week, when the shade was again taken (T4 = total of 2 weeks of bleaching). The Cavit (3M Espe) was removed and the pulp chamber rinsed as previously described, and fresh sodium perborate paste was sealed in the pulp chamber for another week, when the final shade was taken (T5 = total of 3 weeks of bleaching).

**Statistical analysis**

The data were analysed at 5% significance. The t-test was used to analyse intergroup data: the colour stability (ΔE) of the two experimental groups was compared at each time period. ANOVA complemented by the Tukey’s test were used for intragroup comparisons: lightness values at all time periods were compared within each experimental group.

**Results**

Discolouration was observed visually in all specimens after the TAP was sealed in the pulp chamber for 3 weeks. Improvement of the discoloration was noted in all time periods after removal of the TAP and when bleaching was carried out (Fig. 1).

In the intragroup analysis, the values of lightness (L*) were considered at different time periods (Table 1). There was a significant drop in the values of L* between T0 and T1 in both groups (CA – \( P < 0.001 \) and OA – \( P = 0.002 \)). The values rose over the subsequent time periods, increasing significantly in both groups by T5 (CA – \( P = 0.002 \) and OA – \( P = 0.001 \)) and reaching a shade close to the one at T0 at T5 (Fig. 2).

In the intergroup analysis, the overall colour stability was considered (ΔE). The patterns of discoloration and bleaching were similar between CA and OA groups (\( P > 0.05 \)) (Table 1). The only difference found between the two groups was between T1 and T2, when ΔE values were significantly higher in the OA group (\( P = 0.04 \)).

**Discussion**

This study confirmed that TAP discoloured the teeth, as the L* values decreased drastically between T0 and T1. The results corroborate the observations both of clinical reports of discoloration of teeth that underwent revascularization procedures (Kim et al. 2010, Petrino et al. 2010, Dabbagh et al. 2012, Miller et al. 2012) and a laboratory study (Lenherr et al. 2012).

Minocycline is the antibiotic in the TAP responsible for tooth discoloration (Kim et al. 2010). Minocycline is a semisynthetic derivative of tetracycline. Tetracycline has the ability to chelate calcium ions and to be incorporated into teeth, resulting in
The exact mechanism of tetracycline staining, however, is still unknown (McKenna et al. 1999, Sánchez et al. 2004). To avoid discolouration, minocycline could be removed and a combination of only two antibiotics could be used, or the minocycline could be replaced by another antibiotic (Iwaya et al. 2001, Thibodeau & Trope 2007). However, the combination of the three antibiotics has demonstrated to be effective against the variety of endodontic microorganisms in teeth with necrotic pulps (Sato et al. 1993, Hoshino et al. 1996, Nygaard-Østby et al. 1996).

Figure 1 Photographs of specimens in CA and OA groups at the experimental time periods showing the patterns of discolouration and bleaching.

Table 1 Mean values of $L^*$, $a^*$, $b^*$ and $\Delta E$ ($\pm SD$) at T0, T1, T2, T3, T4 and T5

| Group | LT0 | LT1 | $P$ values | aT0 | aT1 | bT0 | bT1 | $\Delta E$
<table>
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<tr>
<td>CA</td>
<td>86.14 ± 4.40*</td>
<td>70.12 ± 5.19b</td>
<td>&lt;0.001</td>
<td>0.64</td>
<td>-1.78</td>
<td>29.40</td>
<td>29.67</td>
<td>17.16 ± 4.56A</td>
</tr>
<tr>
<td>OA</td>
<td>85.25 ± 5.39*</td>
<td>68.15 ± 10.27b</td>
<td>0.002</td>
<td>2.55</td>
<td>0.61</td>
<td>29.52</td>
<td>33.44</td>
<td>18.91 ± 12.40A</td>
</tr>
<tr>
<td>T1 (after 3 weeks with TAP) × T2 (after rinsing off TAP with NaOCl)</td>
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| Group | LT1 | LT2 | $P$ values | aT1 | aT2 | bT1 | bT2 | $\Delta E$
| CA    | 70.12 ± 5.19* | 71.05 ± 5.69a | 0.995 | -1.78 | -1.30 | 29.67 | 30.66 | 2.78 ± 2.73A |
| OA    | 69.25 ± 11.18* | 69.53 ± 10a | 1.000 | 0.61 | 0.57 | 32.57 | 32.57 | 4.94 ± 1.46B |
| T2 (after rinsing with NaOCl) × T3 (after 1 week with sodium perborate paste) | | | | | | | | |
| Group | LT2 | LT3 | $P$ values | aT2 | aT3 | bT2 | bT3 | $\Delta E$
| CA    | 71.05 ± 6a | 75.74 ± 6.83* | 0.630 | -1.30 | 1.42 | 30.66 | 20.93 | 12.07 ± 4.91A |
| OA    | 69.53 ± 10a | 75.13 ± 8.04* | 0.695 | 0.57 | 1.44 | 32.57 | 19.73 | 14.65 ± 6.22A |
| T2 (after rinsing with NaOCl) × T4 (after 2 weeks with sodium perborate paste) | | | | | | | | |
| Group | LT3 | LT4 | $P$ values | aT3 | aT4 | bT3 | bT4 | $\Delta E$
| CA    | 71.05 ± 6a | 75.74 ± 6.83* | 0.036 | 14.91 | 3.71 | 30.66 | 22.50 | 19.38 ± 6.57A |
| OA    | 69.53 ± 10a | 78.69 ± 7.37* | 0.183 | 0.57 | 3.61 | 32.57 | 20.79 | 15.91 ± 9.43A |
| T2 (after rinsing with NaOCl) × T5 (after 3 weeks with sodium perborate paste) | | | | | | | | |
| Group | LT4 | LT5 | $P$ values | aT4 | aT5 | bT4 | bT5 | $\Delta E$
| CA    | 71.05 ± 6a | 83.63 ± 5.69b | 0.002 | -1.30 | 2.10 | 30.66 | 22.51 | 16.58 ± 6.83A |
| OA    | 69.53 ± 10a | 86.21 ± 8.53* | 0.001 | 0.57 | 4.58 | 32.57 | 21.44 | 21.72 ± 14.21A |
| T0 (baseline) × T5 (after 3 weeks with TAP) | | | | | | | | |
| Group | LT0 | LT5 | $P$ values | aT0 | aT5 | bT0 | bT5 | $\Delta E$
| CA    | 86.14 ± 4.40* | 83.63 ± 5.69* | 0.952 | 0.64 | 2.10 | 29.40 | 22.51 | 9.53 ± 4.27A |
| OA    | 85.25 ± 5.39* | 86.21 ± 8.53* | 1.000 | 2.55 | 4.58 | 29.52 | 21.44 | 10.45 ± 4.65A |

Intragroup analyses of mean values of $L^*$ are shown in lines (ANOVA, $P < 0.05$, significant differences indicated by different lower case letters). Intergroup comparisons of mean values of $\Delta E$ are shown in $\Delta E$ column ($t$-test, $P < 0.05$, significant differences indicated by different capital letters).

discolouration. The exact mechanism of tetracycline staining, however, is still unknown (McKenna et al. 1999, Sánchez et al. 2004). To avoid discolouration, minocycline could be removed and a combination of only two antibiotics could be used, or the minocycline could be replaced by another antibiotic (Iwaya et al. 2001, Thibodeau & Trope 2007). However, the combination of the three antibiotics has demonstrated to be effective against the variety of endodontic microorganisms in teeth with necrotic pulps (Sato et al. 1993, Hoshino et al. 1996, Nygaard-Østby et al. 1996).

T1 and T2 were compared to see whether just the standard procedure of rinsing off the TAP with NaOCl
was sufficient to improve the discolouration. \( \text{NaOCl} \) itself is a bleaching agent as it is broken down into chlorine and oxygen, promoting bleaching of dentine (Kashima-Tanaka et al. 2003). Despite this, the sole action of \( \text{NaOCl} \), in combination with a short rinsing time, was not enough to bleach the teeth. In addition, the oxidation reaction may not have been powerful enough to do so either. There is one published case report of a central incisor that underwent revascularization and 6 months later, internal bleaching was successfully performed to remove cervical discoloration caused by the TAP (Miller et al. 2012), but no controlled study has yet assessed whether bleaching procedures could reverse discoloration caused by the TAP. Bleaching with sodium perborate paste, mimicking the walking bleach technique, for all of the time periods assessed (1, 2 or 3 weeks), improved the discoloration of the teeth when compared to rinsing with \( \text{NaOCl} \) only, with proximity in shade to the baseline reached at T5. When in contact with moisture, sodium perborate will slowly decompose into sodium metaborate, hydrogen peroxide and singlet oxygen, which will bleach the dentine by a simple oxidation-reduction reaction (Abbott & Heah 2009). Clinically, the walking bleach technique is a simple procedure with results that have a high patient acceptance (Gupta & Saxena 2014).

Teeth are subjected to several conditions that may change their structure and physiology, such as age, caries and different types of wear and restorative procedures. Changes take place intratubularly with deposition of tertiary dentine, leading to a reduction in the permeability of dentine (Mjor 2009). In this study, teeth with both closed and open apices were used to assess whether there would be differences in their patterns of discolouration and bleaching. It was expected that the teeth in the OA group would discolor more intensely and bleach at a faster rate than the teeth in the CA group due to potentially larger dentinal tubules present in younger teeth in the OA group. Although teeth in the OA group tended to be more intensely discoloured at T1 and bleached more at T5 than teeth in the CA group, surprisingly, significant differences between the two groups in their \( \Delta E \) values were only found at the T1–T2 time periods (the time at which the TAP was rinsed off with \( \text{NaOCl} \)). Clinically, it can be expected that teeth with open apices will bleach at the same rate as teeth with closed apices when using the walking bleach technique.

Teeth in the OA group exhibited a higher value of \( L^* \) at T5 when compared to T0, whilst the teeth in the CA group exhibited a lower value of \( L^* \) at T5 when compared to T0. This may indicate that teeth with closed apices may need more bleaching time than teeth with open apices. Perhaps if the specimens in the OA group had larger apical widths, differences between the two groups would have been found.

In this study, spectrophotometry based on the CIE \( L^*a^*b^* \) system was used to assess changes in shade. The advantage of this system is that the shade differences are expressed numerically, and these can be related to visual perception and clinical significance (Douglas et al. 2007). Values of \( L^* \), \( a^* \) and \( b^* \) were collected, as they were needed in the formula used to calculate the \( \Delta E \), which represents the constant difference in colour, regardless of the location in the colour space (Figueiredo et al. 2014).
Values at different time periods have to be collected to calculate a Δ; a Δ value is calculated by subtracting the final data from the initial data in each time period. It was possible to calculate the constant difference in colour (ΔE) for intergroup comparisons, where characteristics were compared between two time periods. However, only values of L* (lightness) were considered for intragroup comparisons as a Δ could not be calculated. As the darkening and lightening patterns were the ones of biggest concern, this does not represent a limitation.

For the sake of interpretation, it is important to understand that, according to the CIE L*a*b* system, the constant difference in colour (ΔE) with values above 3.7 are considered an easily visible difference (Ruyter et al. 1987, Figueiredo et al. 2014). In the present study, most ΔE values in both groups were above 3.7, which are considerable values that were easily perceptible. The only lower ΔE values found were shown between T1 and T2, when the simple rinsing off of the TAP with NaOCl did not alter the shade of the samples (CA = 2.76; OA = 4.94).

The literature indicates that approximately 51% of reported revascularization cases were treated with an antibiotic paste as the intracanal medicament, including TAP (Diogenes et al. 2013). This present study has shown that discolouration caused by TAP can be reversed to a shade similar to the baseline one.

**Conclusions**

Under the conditions of this study, TAP discoloured the tooth structure and the same teeth could be bleached with sodium perborate paste. In general, teeth with closed and open apices had the same rates of discoulouration and bleaching.

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**References**


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