Tooth discoloration induced by endodontic materials: a laboratory study

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Abstract

Aim To investigate the discoloration potential of endodontic materials using a bovine tooth model.

Methodology Two hundred and 10 dentine-enamel cuboid blocks (10 x 10 x 3.5 mm) were prepared out of the middle thirds of bovine tooth crowns. Standardized cavities were prepared in the walls of the pulp chamber leaving 2 mm of enamel and dentine on the labial wall of the crown. The specimens were randomly assigned to 14 groups (n = 15). Endodontic materials were placed into the cavities as follows: group A: empty, group B: blood, group C: calcium hydroxide, group D: Apexit, group E: Ultracal XS, group F: Listerm, group G: triple antibiotic paste (3Mix), group H: grey MTA, group I: MTA + blood, group J: white MTA, group L: Portland cement (PC), group M: PC + blood and group N: AH Plus. The cavities were sealed with composite and stored in water. Standardized colour measurement (VITA Easyshade compact) was performed at the following intervals: prior to (T0) and after placement of the filling (T1), 1 week (T2), 1 month (T3), 3 months (T4), 6 months (T5) and after 1 year (T6). Colour change (DE) values were calculated. A two-way analysis of variance was used to assess significant differences between the endodontic materials. The mean values of all groups were compared using the Tukey multiple comparison test (α = 0.05).

Results Significant differences were detected amongst the experimental groups after 12 months (P < 0.0001). The lowest colour change values were observed in the groups N (AH Plus, 3.2 ± 1.5), A (empty, 3.8 ± 1.4), L (PC, 4.1 ± 1.7), C (calcium hydroxide, 4.7 ± 1.5), E (Ultracal XS, 5.1 ± 1.9) and J (WMTA, 7.9 ± 6.7). The most discoloration was measured in groups G (3Mix, 66.2 ± 9.9) and F (Ledermix, 46.2 ± 11.6). PC showed the best colour stability amongst the Portland cement-based materials; however, when contaminated with blood (group M), a significantly higher DE value (13.6 ± 4.2) was detected (P = 0.032).

Conclusion Materials used in endodontics may stain teeth. Therefore, the choice of material should not rely solely on biological and functional criteria, but also take aesthetic considerations into account.

Keywords: bovine tooth model, discoloration, endodontic materials, sealer, staining.

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Introduction
Tooth discoloration induced by endodontic materials is a common finding and may impair the aesthetic outcome of endodontically treated teeth (van der Burgt et al. 1986a,b). A progressive discoloration is suggested to be primarily a result of materials ingressing into
dentinal tubules (van der Burg et al. 1986a,b). However, it has been shown that a visible crown discoloration may not necessarily be associated with tubule penetration and may be caused by material remnants in the pulp chamber, which get darker over time and transmit through the hard tissues (Davis et al. 2002).

The staining ability of several endodontic materials including Walkhoff’s paste, Grossman’s cement, zinc oxide eugenol, endomethasone and N₂ has been demonstrated (Gutierrez & Guzman 1968, van der Burg et al. 1986a,b). However, most of these materials are no longer used.

Limited data are available on the staining ability of recent endodontic materials. Most studies that focused on root canal sealers concluded they stained teeth. This was shown in laboratory studies for AH26, Kerr Pulp Canal Sealer, Roth 801, Sealapex, Endofill, Tubliseal, zinc oxide eugenol, Apatite Root Canal Sealer, Cavizol, AH Plus and EndoREZ (Parsons et al. 2001, Davis et al. 2002, Partovi et al. 2006, Elkhazin 2011). The discolouration potential of Ledermix has been evaluated ex vivo (Kim et al. 2000a,b) and in a recent clinical study (Day et al. 2011). In the same three studies, calcium hydroxide pastes were used as controls. It was concluded that, compared with Ledermix, calcium hydroxide may cause only minor but measurable staining. However, there are various formulations of calcium hydroxide available with different constituents, which have been added to the powder to improve properties such as antibacterial action, radiopacity, flow and consistency (Fava & Saunders 1999). These additives may affect the staining ability of the pastes.

Several case reports reveal discoloration produced by MTA. However, there is only one study documenting severe discoloration caused by MTA when the material was used as a pulpotomy agent in primary molars (Naik & Hegde 2005).

Although manufacturers such as Medcem GmbH (Weinfelden, Switzerland) claim that a better colour stability is achieved when using Portland cement (PC) instead of MTA, there are no studies available to prove that statement.

Discoloration after canal medication with triple antibiotic pastes (3Mix), as used in the field of regenerative endodontics, has only been scarcely mentioned in the literature (Trope 2010). Only one laboratory experiment proved clear evidence of tooth discoloration after use of the paste (Kim et al. 2010).

In summary, no systematic approach has been used to evaluate and compare the discoloration induced by materials, which are used in endodontics. This study was undertaken to develop a new model for the assessment of tooth discoloration and to investigate the staining ability of endodontic materials.

The tested hypotheses were (i) there is no difference in discoloration amongst the tested materials after 12 months and (ii) all materials show a similar progression of discoloration over time.

Material and methods

Specimen preparation

Two hundred and 10 bovine incisors were extracted, cleaned and stored in water at room temperature. Following the removal of the roots, the labial surface of each tooth was cleaned meticulously with scalers. A cuboid enamel-dentine block (10 × 10 mm) was prepared from the middle third of each crown using a diamond-coated disc (Intensiv SA, Grancia, Switzerland). The height of each block was standardized at 3.5 ± 0.1 mm, measured with a calliper (Iwanson, Ustomed, Tuttlingen, Germany). A cylindrical-shaped hole with the diameter of 2.5 mm was drilled with a form-congruent bur (Dentsply Maillefer, Ballaigues, Switzerland) in the middle of each specimen to leave 2 mm of the labial tooth structure (Fig. 1). The specimens were placed in 1% sodium hypochlorite for

Figure 1 Schematic showing the tooth piece cut out of the middle third of the crown of a bovine incisor. The spectrophotometer is held on to the tooth piece.
30 min and, after drying with air, placed in 20% EDTA (lege artis, Detthausen, Germany) for two additional minutes to remove the smear layer. After a final 3 min in sodium hypochlorite, the specimens were stored in tap water.

The specimens were randomly assigned to 14 groups (n = 15), and different endodontic materials (Table 1) were placed into the cavities. The materials were prepared as indicated by the manufacturer’s guidelines/recommendations. In group G, a triple antibiotic paste including metronidazole, minocycline and ciprofloxacin was produced according to the composition and mixing instructions described by Trope (Trope 2008). In groups L, K and M, 1.5 μL of bovine blood was placed on top of the material with a pipette (0.5–10 μL; Eppendorf AG, Hamburg, Germany) to simulate a typical clinical situation in which calcium silicate-based materials are in contact with vital and vascularized tissue. This situation was not simulated for the root canal dressings and sealers because their application implies that vital tissues have been removed from the root canal.

The cavities were sealed with a self-adhesive resin material (RelyX Unicem; 3M ESPE, Seefeld, Germany). Polymerization was initiated with a LED curing light (Smart Lite PS Series, Dentsply International, York, PA, USA) for 20 s.

Every specimen was placed into a single tube with tap water (Standard Micro Test Tube 3810; Eppendorf AG, Hamburg, Germany). The tubes were stored at room temperature and kept in the dark during the first 3 months. During the following period and up to 12 months, the specimens were exposed to indirect sunlight until the end of the experiment.

Colour determination

Colour measurements were taken in a dark room with a spectrophotometer (VITA Easyshade® compact; VITA Zahnfabrik, Bad Säckingen, Germany) under standardized conditions in a custom-fabricated measuring station (Fig. 2). The station consisted of a wooden board with a fixed lamp, a carrier for the specimens and the VITA Easyshade® Compact unit. The instrument was calibrated before the measurement in each group.

Seven sessions of colour measurements were obtained at the following intervals: prior (T0 = baseline).

| Table 1 Materials used in the different experimental groups and their compositions |
|---|---|---|---|
| Group | Material | Details/composition | Manufacturer |
| A | Empty | Negative control group | – |
| B | Blood | Positive control group | – |
| C | Calcium hydroxide | Pure calcium hydroxide mixed with saline | Produits Dentaires, SA, Vevey, Switzerland |
| D | ApexCal | Injectable calcium hydroxide paste containing bismuth carbonate as radiopacifier | Ivoclar Vivadent, Schaan, Liechtenstein |
| E | Ultracal XS | Injectable calcium hydroxide paste containing barium sulphate as radiopacifier | Ultradent Products, South Jordan, Utah, USA |
| F | Ledermix | Glucocorticosteroid-antibiotic root canal dressing (demeclocycline-HCl 3.2% and triamcinolone acetonide (1%), in a polyethylene glycol base) | Riemser Arzneimittel, Greifswald, Germany |
| G | 3Mix | Triple antibiotic mixture (ciprofloxacin, metronidazole and minocycline) in a macrogol and propylene glycol carrier | Ciprofloxacin (Sandoz, Holzkirchen, Germany), Metronidazole (Sanofi-Aventis, Frankfurt Germany), Minocycline (Spirig Pharma AG, Egerkingen, Switzerland) |
| H | GMTA | Grey mineral trioxide aggregate, containing bismuth oxide as radiopacifier. | ProRoot MTA, Dentsply, DeTrey, Konstanz, Germany |
| I | GMTA + blood | White mineral trioxide aggregate (similar to GMTA, but lower amounts of iron, aluminium and magnesium) | ProRoot MTA, Dentsply, DeTrey, Konstanz, Germany |
| J | WMTA | Portland cement (similar to MTA but absence of bismuth ions and presence of potassium ions | Medcem GmbH, Weinfelden, Switzerland |
| K | PC | Epoxy-amine resin-based root canal sealer, zirconium oxide as radiopacifier | Dentsply, DeTrey, Konstanz, Germany |

PC, Portland cement.
and after placement of the filling (T1), after 1 week (T2), 1 month (T3), 3 months (T4), 6 months (T5) and 12 months (T6). To prevent optical changes caused by dehydration, the excess water was removed briefly by air-drying for 1 s. The entire measurement was completed within 5 s for every specimen, and each specimen was measured once. The CIE L*a*b* data were collected and further analysed.

**Statistical analysis**

For each specimen, colour change (ΔE) values were calculated with the following formula.

\[
\Delta E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}; \Delta L^* = L_1^* - L_0^*; \Delta a^* = a_1^* - a_0^*; \Delta b^* = b_1^* - b_0^*.
\]

For each group, the means of the ΔE values were calculated at the given time intervals.

A two-way analysis of variance was used to assess significant differences between the tested endodontic materials. The mean value of all groups were compared using the Tukey multiple comparison test (α = 0.05).

**Results**

The ΔE values at several time intervals are presented in Fig. 3. The most severe discoloration after 12 months was caused by Ledermix and 3Mix. However, during the period of dark storage (first 3 months), Ledermix did not reveal a significant difference compared with baseline (P = 0.3057), whilst a highly significant difference was observed for 3Mix (P < 0.0001). Progressive discoloration in terms of a significant difference between baseline and 12 months was also observed for ApexCal (P < 0.0001), WMTA + blood (P = 0.0146) and PC + blood (P = 0.0206).

GMTA showed severe discoloration from the time of placement (T1). After a slight increase during the first week, the mean ΔE value seemed to remain stable at this high level.

ΔE mean values and standard deviations after 12 months are given in Table 2. At this time interval, PC caused the least discoloration amongst the Portland cement-based materials when no blood was added, the difference being statistically significant compared with GMTA (P < 0.0001) but not compared with WMTA (P = 0.9283).

**Discussion**

**Study design and limitations**

This study investigated the potential of endodontic materials to induce discoloration in a new experimental set-up using a bovine tooth model. The majority of previous studies have used human teeth to assess the degree of staining. However, the significant variability in tooth morphology combined with small sample sizes may influence the results considerably. To overcome these limitations, the present laboratory model used standardized bovine tooth pieces with a similar shape and allowed for the precise measurement of discoloration over time.
and thickness. Furthermore, the colour analysis was conducted under standardized conditions in a custom-fabricated measuring station to ensure that the consecutive measurements were taken at the same region in each sample.

For colour determination, the Vita Easyshade Compact Device was chosen because of its high data stability and excellent repeatability (Lehmann et al. 2010). Even though deviations from the spectrophotometric reference may occur with most commercially available devices, this discrepancy may not be relevant for this study, because the assessment of colour changes and not the determination of the exact tooth colour were essential.

Although bovine root dentine has a significantly higher tubule density than human samples, coronal dentinal layers do not differ significantly in terms of density or diameter of the tubules (Schilke et al. 2000). This suggests that bovine incisor crowns may represent suitable substitutes for human teeth in laboratory studies dealing with tooth discoloration.

The severity of tooth discoloration depends on whether the smear layer is removed or not (Davis et al. 2002). It has been reported that the smear layer can markedly reduce the permeability of dentine (White et al. 1987). In studies performed without the removal of smear layer, tooth discoloration was less evident or took longer (Parsons et al. 2001, Davis et al. 2002). Recent protocols for root canal irrigation recommend the removal of the smear layer to facilitate the disinfection of the root canal system (van der Sluis et al. 2007). To reproduce a realistic clinical situation and to provide optimal penetration of the endodontic materials into the dentinal tubules, the smear layer was eliminated in the present study.

Despite the standardized experimental set-up, the present model has limitations in fully imitating the clinical situation. Interaction of the endodontic material with salivary components and bacteria may occur if there is leakage at the restoration margins. This may lead to different staining mechanisms in vivo. Furthermore, placing medicaments or sealers intentionally for up to 12 months inside the tooth crown may exaggerate the clinical situation in which materials in the access cavity are removed before the final restoration is performed.

The present survey revealed significant differences between the endodontic materials with regard to their ability to discolor teeth. Furthermore, the materials showed a different progression of discoloration over time. Thus, both hypotheses had to be rejected.

### Discoloration by Portland cement-based materials

Portland cement-based materials have gained much popularity in endodontics because of their biocompatibility and good sealing properties (Parirokh & Torabinejad 2010, Torabinejad & Parirokh 2010).

Amongst these materials, in the present study, the best colour stability could be achieved with PC even though the difference was not significant compared with WMTA after 12 months. PC differs from the MTA by the absence of bismuth ions and presence of potassium ions (Song et al. 2006). According to Steffen and van Waes (2009), bismuth oxide, which has been added to PC to increase radiopacity, is a possible factor responsible for the discoloration of teeth treated with MTA. After contamination of the specimens with blood, all Portland cement-based materials showed an increased discoloration. Namazikhah et al. (2008) demonstrated that the microstructure of the materials shows pH-dependent porosities. These porosities may uptake blood components and may be responsible for the observed discoloration. This is of clinical relevance because Portland cement-based materials are usually placed in direct contact to vital, vascularized tissue. Thus, the development of biocompatible materials with a reduced porosity level may be beneficial.

### Discoloration by triple antibiotic pastes

The most severe discoloration was seen when the triple antibiotic paste containing ciprofloxacin, metronidazole

<table>
<thead>
<tr>
<th>Group</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>G (3Mix)</td>
<td>66.2 ± 9.9 a</td>
</tr>
<tr>
<td>F (Ledermix)</td>
<td>46.2 ± 11.6 b</td>
</tr>
<tr>
<td>H (GMTA)</td>
<td>21.2 ± 7.2 c</td>
</tr>
<tr>
<td>I (GMTA + blood)</td>
<td>20.9 ± 5.5 c</td>
</tr>
<tr>
<td>B (blood)</td>
<td>20.1 ± 4.4 cd</td>
</tr>
<tr>
<td>D (ApexCal)</td>
<td>13.9 ± 10.2 cde</td>
</tr>
<tr>
<td>M (PC + blood)</td>
<td>13.6 ± 4.2 cde</td>
</tr>
<tr>
<td>K (WMTA + blood)</td>
<td>12.6 ± 5.2 def</td>
</tr>
<tr>
<td>J (WMTA)</td>
<td>7.9 ± 6.7 mg</td>
</tr>
<tr>
<td>E (Ultracal XS)</td>
<td>5.1 ± 1.9 h</td>
</tr>
<tr>
<td>C (calcium hydroxide)</td>
<td>4.7 ± 1.5 g</td>
</tr>
<tr>
<td>L (PC)</td>
<td>4.1 ± 1.7 f</td>
</tr>
<tr>
<td>A (empty)</td>
<td>3.8 ± 1.4 e</td>
</tr>
<tr>
<td>N (AH Plus)</td>
<td>3.2 ± 1.5 g</td>
</tr>
</tbody>
</table>

PC, Portland cement.

Levels not connected by same superscript are significantly different.

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Lenherr et al.  Discoloration from endodontic materials

and minocycline was used. The specimens in this group became almost black after 12 months. Discoloration after canal medication with the triple antibiotic paste has scarcely been mentioned in the literature (Trope 2010). Kim et al. (2010) identified minocycline as the cause for discoloration in vitro. Furthermore, the latter study demonstrated that sealing the dentine wall with a bonding agent prior to application of the paste could reduce the overall colour change, without being able to prevent it. Even though the triple antibiotic paste originally introduced by Hoshino et al. (1996) reliably eradicates bacteria from infected root canals setting the conditions for subsequent re-vascularization in the field of regenerative endodontic procedures (Trope 2008), it may cause severe aesthetic problems. Thus, future research should focus on alternative mixtures with a substitute for minocycline. Trope (2010) showed that with Arestin as a substitute discoloration could be markedly reduced but not prevented.

As a comparable alternative a prefabricated triantibiotic mixture with cefuroxim as a substitute for minocycline is available (TreVitaMix; Medcem GmbH). However, there are no studies available neither regarding the disinfection potential of the mixture in the root canal, nor its discoloration ability.

**Discoloration by Ledermix**

Ledermix has regained attention in the field of dental traumatology. Placed as an intracanal medication after severe luxation injuries, it has the potential to inhibit inflammatory root resorption (Pierce et al. 1988, Bryson et al. 2002, Wong & Sae-Lim 2002). However, in the present study, severe and increasing discoloration occurred in the Ledermix specimens especially after the specimens had been exposed to indirect sunlight. This is in accordance with other laboratory (Kim et al. 2000a,b) and clinical studies (Day et al. 2011). From a clinical aspect, it may not be possible to completely avoid contamination of the coronal dentine with the medicament or to meticulously clean the surfaces after placement. Thus, alternative nonstaining alternatives are needed. This may be Odontopaste (Australian Dental Manufacturing, Kenmore Hills, Australia), a recently released alternative with substitution of the tetracycline component by clindamycin (Athanassiadis et al. 2011). Furthermore, as the inhibiting effect of Ledermix on external root resorption after severe dislocation injuries is primarily attributed to the corticosteroid component (Chen et al. 2008, Kirakozova et al. 2009), a corticosteroid dressing may be a reasonable alternative in dental traumatology.

**Discoloration by AH Plus sealer**

AH Plus, the only sealer tested in this study, seemed to exhibit satisfactory colour stability, presumably owing to the stable radiopacifier. Zirconium oxide has a high radiopacity but is not known to be involved in discoloration. However, the present results are in contrast to the findings of Elkhazin (2011) who showed distinct discoloration induced by AH Plus after 6 weeks, which tended to decline after 8 weeks. The present study also revealed a greater colour change during the first 3 months and declining ΔE values during the remaining observation period up to 12 months.

**Discoloration by calcium hydroxide**

The calcium hydroxide dressings varied in their discoloration ability. Pure calcium hydroxide and Ultracal XS did not show any discoloration or difference to the negative controls at any time. Interestingly, the ApexCal specimens showed an increase in their ΔE values in the second half of the observation period. The difference to all the other calcium hydroxide materials was statistically significant after 12 months. As bismuth carbonate is part of the chemical composition of ApexCal (22 weight per cent, according to the manufacturers specification), a discoloration produced by bismuth ions may explain this finding.

A recently published randomized controlled clinical trial on replanted teeth demonstrated that even Ultracal XS produced measurable colour changes (ΔE change in colour = 3.0) in a clinical situation. However, only 1 of 12 patients was concerned about the colour of the tooth compared with 7 of 10 patients in the Ledermix group (Day et al. 2011). In the laboratory tests for this study, the mean ΔE change in colour for the Ultracal XS group was 5.1. In a clinical situation, a minor colour mismatch between ΔE 2.6 and 3.7 is sufficient to be identified by a dentist. However, a difference of ΔE 5.5–6.8 is needed to classify the discoloration as unacceptable and recommend further treatment (Day et al. 2011).

**Conclusions**

Materials used in endodontics may stain teeth. Therefore, the choice of material should not rely solely on
biological and functional aspects, but also take aesthetic considerations into account. Underlying mechanisms of tooth discoloration by endodontic materials should be investigated in further research projects and nonstaining alternatives developed.

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