Discoloration of Teeth after Avulsion and Replantation: Results from a Multicenter Randomized Controlled Trial

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Abstract

Introduction: There is evidence to suggest that Ledermix, placed as an intervisit root canal dressing, might improve periodontal healing after replantation of avulsed teeth. As a part of a multicenter randomized controlled trial, we aimed to compare the effect of 2 root canal medicaments, Ledermix and Ultracal XS, on the discoloration of replanted teeth. Methods: Discoloration was investigated by using 3 methods: patient satisfaction with the color of replanted teeth, clinical photographs taken at baseline and 12-month reviews, and estimation of color change by using CIELAB scores for baseline and 12-month photographs. Results: Twenty-two patients (27 teeth) were recruited. Ten patients (12 teeth) were randomized to the Ledermix group and 12 patients (15 teeth) to the Ultracal XS group. At 12 months, 8 patients were concerned with the discoloration of their teeth. Seven came from the Ledermix group and 1 from the Ultracal XS group. This difference was significant (Fisher exact test, \( P = .009 \)). Standardized photographs were taken for the patients recruited at one center only (17 patients). There was significant discoloration of teeth from baseline with Ledermix, causing a darkening and gray-brown discoloration (mean change from baseline to 12 months, \( L^* = -5.1, a^* = 0.3, b^* = -1.2, \) and \( \Delta E = 8.1 \)) and Ultracal XS, causing a yellowing and lightening of teeth (\( L^* = 1.9, a^* = 0.3, b^* = 3.3, \) and \( \Delta E = 5.4 \)). There was a significant difference for the \( L^* \) and \( b^* \) variables (independent t test) between the 2 groups. Conclusions: Both root canal medicaments cause discoloration, with Ledermix proving less acceptable to patients. (J Endod 2011;37:1052–1057)

Key Words

Avulsion, discoloration, Ledermix, multi-center, non-setting calcium hydroxide, randomized controlled trial

Discoloration of teeth after replantation after avulsion is recognized. Ebeleseder et al (1) reported a prevalence of severe discoloration of 33% for immature teeth that had been given the opportunity to revascularize. Another study reported that 27 of 28 teeth showed adverse color changes ranging from grayish to bluish-black (2).

Although color change has been reported as a consequence of dentoalveolar trauma by a number of authors, the mechanisms are not fully understood (3, 4). It does appear that different colors represent different clinical entities, some of which represent pulpal damage with subsequent healing, whereas others indicate pulpal necrosis. Furthermore, discoloration can occur as a result of materials used for subsequent endodontic treatment (5, 6).

Ledermix (Henry Schein UK [Holdings Limited]) has demonstrated increased rates of periodontal healing in a number of animal studies (7–11) for avulsed and replanted teeth. Ledermix is a water-soluble paste containing 1% triamcinolone, a corticosteroid, and 3% demeclocycline, a tetracycline. Two in vitro studies have investigated the effect of discoloration with Ledermix in immature (12) and mature roots (13). These studies showed that Ledermix caused a dark, gray-brown discoloration of teeth that was exacerbated by sunlight and immature root development. When Ledermix was restricted to the root with no material allowed into the crown; the degree of discoloration was less severe.

After avulsion and replantation in closed apex teeth, current guidelines (14) advocate that pulp extirpation and disinfection are instigated at day 7–10. The reason for this is that the recommended intracanal medicament, non-setting calcium hydroxide, showed significant detrimental outcomes to periodontal healing in comparison to a more inert gutta-percha root filling if placed earlier (15, 16). There are a number of studies in the literature with respect to color change after application of calcium hydroxide (12, 13, 17). It has also been noted that a more significant yellow color change was associated with immature rather than mature teeth (12).

Color change in teeth is difficult to measure because of many different factors. These include the effect of adjacent tissues (eg, gingivae), the area of tooth being measured, surface imperfections and defects, lighting conditions, angulations of the tooth surface, and dehydration. Consequently, a number of methods have been established and tested to determine tooth color or color changes over time (18). The simplest method is evaluating the tooth next to a shade guide of different tooth colors to monitor changes. Other methods include photographing the teeth in standardized conditions by using an adjacent universal gray block or the use of
Methods

This article reports on one of the secondary outcome measures for a multicenter RCT designed to study outcomes of teeth, replanted after avulsion, in which the initial root canal medicament was either Ultracal XS (a commercially prepared non-setting calcium hydroxide paste with high viscosity; Ultradent Products Inc, South Jordan, UT) or Ledermix. The primary outcome measure was periodontal healing; this has been reported in a different article (21). Before the study could commence a number of research approvals were secured, including ethical approval (04/Q1205/145), Medicines and Healthcare products Regulatory Agency (MHRA) (CTA 16767/0203/001-0001), European Union Drug Regulating Authorities Clinical Trials (EudraCT) number (2004-005188-12), and registration of the trial with International Standard Randomized Controlled Trial Number (ISRCTN) (58467151). Informed consent was obtained from all patients recruited to the study. All avulsed teeth were replanted within 60 minutes. The trial medications were placed in the root canal within 10 days (baseline) and remained in situ for 60–90 days. Both groups were then subject to an additional 3 months of intracanal dressing with Ultracal XS before obturation with gutta-percha and Tubliseal (SybronEndo, Orange, CA) at 6 months. Patients were reviewed at 12 months. At this appointment the patients were asked whether they were happy with the color of the avulsed and replanted tooth or teeth.

All patients who had care provided at Leeds Dental Institute had photographic records taken at each clinical visit to monitor discoloration. These were taken in the photography department by trained medical photographers with a Fujifilm (Tokyo, Japan) Finepix S3 Pro camera with 105-mm Micro Nikon (Tokyo, Japan) f2.8 lens and sigma ring flash, EM-140DG. Although attempts were made to standardize the way all photographs were taken, there was some variability in each image, depending on the camera used, the lighting, the photographer, or the photographic view taken.

To facilitate comparison between photographs and then quantify any color change, dedicated software was developed by using MathWorks (Natick, MA). The software, which is described in more detail in Figure 2, calculated the red-green-blue (RGB) and L*, a*, b* values for 2 areas of interest, after it had first corrected the color of the second photograph to that of the first. The program then displayed these values for the reference (baseline) and corrected (12-month) photographs, together with the change in color, CIELAB ΔE.

The dedicated software (Fig. 2) was also used to estimate the difference in color between 2 different teeth in the same photograph. By fixing the ratio for color correction so that none occurred, the same photograph could be imported into the upper left, upper right, and lower right panels. This then allowed the avulsed tooth and contralateral nonavulsed tooth to be compared in the 12-month photograph.

The L*, a*, b* values for the baseline and 12-month photographs (including contralateral nonavulsed teeth) together with the CIELAB ΔE were recorded onto an SPSS spreadsheet (Statistical Package for the Social Sciences, version 16.1; SPSS Inc, Chicago, IL). Continuous data were assessed for whether they were normally distributed by using a Shapiro-Wilk test, and this determined whether an independent t or Mann-Whitney U test was used. A summary statistic, change in L*a*b*, was calculated for each avulsed tooth between the baseline and 12-month photographs. The statistical tests were applied to these data. For dichotomous patient satisfaction data, either Pearson χ² or Fisher exact test was used, dependent on the numbers of observations in each cell.

Results

Eight of the 22 patients recruited at all centers were concerned with the color of their avulsed teeth. Seven of these patients had been randomized to the Ledermix group (n = 10). One of these patients was concerned with the color of 1 tooth but not with 2 other replanted teeth. One patient (n = 12) in the Ultracal XS group expressed dissatisfaction with the color. This difference was significant when using a Fisher exact test, whether the patient with multiple teeth in the Ledermix group was included in both groups (P = .009) or excluded (P = .02) from the analysis.

For the patients who were seen at Leeds Dental Institute, samples of the baseline and final 12-month review photographs are shown in Figure 3 before color correction. There was expensive spectrophotometers or colorimeters. All these methods have their disadvantages.

However, apart from shade guides, these other systems have one distinct advantage in that they can transform the results into a numeric color scale, CIE (1976) L*a*b* (abbreviated to CIELAB). CIELAB represents an approximately uniform color space, or globe, with equal distances corresponding to near-identical perceived color differences (18). The advantage of using this system is that for each color, a numeric value is given for 3 coordinates. In addition, where there is a change in color with time or objects of 2 different colors, the change can be calculated for the 3 coordinates (ΔL*, Δa*, Δb*), and the distance between 2 points on the color space can be calculated to give a ΔE (Fig. 1). Two clinical studies (19, 20) investigated the size of the color mismatch needed between teeth, ΔE 2.6 and ΔE 3.7, before this was identified by dentists. A difference of ΔE 5.5 and 6.8 was required for dentists to find the color mismatch sufficiently unacceptable to suggest further treatment to improve it.

The aim of this study was to investigate the discoloration of teeth after application of 2 root canal medicaments for replanted teeth by using a multicenter randomized controlled trial (RCT).

Figure 1. The 3-dimensional color space CIE (1976) L*a*b* (18). For every color a point in this space can be identified by using the 3 color coordinates L*, a*, and b*. A change in color (from point X to X1) can be calculated numerically by the distance between the 2 points ΔE. This value will only give the size of the color change, not the direction. For this the individual ΔL*, Δa*, Δb* would be needed.
a noticeable darkening and graying effect of the teeth in the Leder-
mix group and a yellowing effect in the Ultracal XS group. Further-
more, there was variation in color within each root canal
medicament group.

The change in color between baseline and 12 months was quan-
tified for each tooth by using the dedicated software. Twenty-two teeth
(17 patients) seen in Leeds Dental Institute were evaluated. For 2
patients in the Ledermix group, the data set was incomplete. For 1
patient with 1 avulsed tooth, a baseline and day 60–90 photographs
were all that were available, because this patient failed to attend further
appointments. Therefore, the results from the day 60–90 photograph
were grouped with 12-month data. For the second patient with 1 avulsed
tooth, no baseline photograph was taken; therefore, the data from the
day 60–90 were analyzed with baseline photograph data. Table 1 shows
baseline, 12-month, and change in color for L*, a*, b* in the CIELAB
space and CIELAB $\Delta E$ values. There was a significant difference for
L* ($P = .003$; confidence interval, 2.6–11.3; independent $t$ test) and
b* ($P = .009$; confidence interval, 1.3–7.9; independent $t$ test). The Ul-
tracal XS baseline data for a* were not normally distributed as deter-
mined by the Shapiro-Wilks test for normality. Therefore, for these
data the median and interquartile range are reported. There was no
significant difference for the change in color between the groups as
determined by Mann-Whitney $U$ test. At baseline, day 0–10, there was
no significant difference between Ledermix and Ultracal XS groups
with respect to L*a*b* (independent $t$ test or Mann-Whitney $U$ test, de-
pending on normality of data).

To validate this change in color between the 2 groups, where
a contralateral central incisor that had not been avulsed was available,
the difference in color between the 2 teeth was calculated for the 12-
month review photograph. For this, only 12 teeth (12 patients) were
eligible, with 7 from the Ledermix group and 5 from the Ultracal XS
group. One patient in the Ledermix group had only the day 60–90 photo-
graph, because the patient was lost to follow-up. Therefore, this photo-
graph was used for comparison. This patient and another who had
avulsed canines had their uninjured contralateral canine used for
comparison. The change in color between the 2 teeth is displayed in
Table 2. There was a significant difference for L* ($P = .007$; Mann-
Whitney $U$ test) variable and CIELAB $\Delta E$ value ($P = .045$; independent
$t$ test; confidence interval, 0.12–8.82). For the contralateral nonavulsed
tooth there was no significant difference between the Ledermix and

Figure 2. Dedicated software was used to correct the color of photographs taken at different visits to then calculate whether there was any change in color between
the 2 time points or between the 2 treatment groups. The picture in the top left panel was the original photograph taken at the initial visit (baseline). The picture in
the top right panel was a picture taken at a subsequent review visit (12-month). Before any change in color could be established between the two, the color of the
12-month photograph was corrected to that of the baseline photograph. This was carried out by identifying a color stable, chromatically neutral, reference point in
both photographs, for example, an uninjured adjacent tooth. In this case part of tooth #11 was used. The program calculated the XYZ values (this is an intermediary
space between RGB and CIELAB) for this reference point between the 2 photographs and then used this ratio to correct the color in the second photograph back to
that of the first. The color-corrected 12-month picture was then shown in the lower right panel.

![Image of software interface for color correction](image-url)
Ultracal XS groups when using appropriate statistics (independent $t$ test or Mann-Whitney $U$ test, depending on normality of data).

**Discussion**

This study used 3 methods to assess discoloration: patient satisfaction with the color of replanted teeth, clinical photographs taken at baseline and 12-month reviews, and estimation of color change by using CIELAB scores for baseline and 12-month photographs.

It is important to investigate whether children were happy with the color of their avulsed and replanted teeth. In adult studies, dissatisfaction with the color of their teeth is a common complaint and reason to seek dental care (22). This was especially true for women and younger people (23). For children, the consequences of poor dental appearance or untreated dental trauma led to negative social judgments being made by their peers (24, 25). Dental features have been shown to be one of the most common causes for teasing at school (26). Consequently, for children and adolescents involved in this multicenter RCT, the appearance of their teeth was important, and for some this might have influenced their own self-perception and sense of how others perceived them. Figure 3 shows examples of the difference in baseline and 12-month photographs for both medicaments. Patient dissatisfaction with the color of their replanted teeth was more common in the Ledermix group. This was a significant finding, despite the small number of patients recruited. What is interesting is that some children with minor discoloration were unhappy with the color of their teeth, when others with more marked discoloration were not. This phenomenon has been investigated with children who have enamel opacities. Different
children’s perceptions of themselves and their friends varied according to the importance they placed on different domains such as physical attributes or personality (27).

Although there are many techniques to monitor color change, these frequently use expensive equipment and require a fixed jig for each patient to ensure the assessment made is from an identical point. Therefore, the method studied used standardized photographs taken by professional medical photographers to be used. This minimized the time required for this part of the clinical visit, when other clinical and radiographic special tests were required in addition to providing the treatment. The methodology used to correct the photographs taken in medical photography allowed quantification of color change, in comparison to simply looking at the baseline and 12-month photographs. There were 2 processes when using the dedicated software, which could account for error. The first was the identification of a color stable point on an uninjured tooth that was used to standardize the color in the 12-month photograph to that of the baseline photograph. The second was the failure to identify the same area of interest in both photographs. The size of each error was estimated and therefore, the estimation of effect would be more accurately calculated by using multileveled modeling statistics. However, this is a time-consuming process, and the size of the effect, which is clearly visible in Figure 3, might be able to compensate for the simple statistics used. These statistical issues do not extend to the comparison with the nonavulsed contralateral tooth because these 3 patients had no such tooth to make the comparison and were therefore excluded from the analysis.

For the Ledermix group there was a mean change in color from baseline to 12 months of $L^* = -5.1$ (the tooth got darker), $a^* = 0.3$ (tooth got redder), and $b^* = -1.2$ (the tooth got bluer). For all these changes the standard deviation was larger than the mean change, showing a wide range in color changes seen. Values from in vitro work for both mature and immature teeth confirmed this direction and the approximate magnitude of color change including the insignificant finding for $a^*$ variable (12, 13). The methodology used, multicenter RCT, was the most robust way of proving an association because potential confounders, either known or unknown, had a random chance of being allocated to either of the study groups. Comparison of baseline photographs taken within 10 days of the injury of the nonavulsed contralateral tooth showed no significant difference between the groups, which demonstrates further the benefit of randomization. Which constituent within Ledermix is the cause of the discoloration is still unproven, because no results with regard to color were provided by the one animal study that therefore, the estimation of effect would be more accurately calculated by using multileveled modeling statistics. However, this is a time-consuming process, and the size of the effect, which is clearly visible in Figure 3, might be able to compensate for the simple statistics used. These statistical issues do not extend to the comparison with the nonavulsed contralateral tooth because these 3 patients had no such tooth to make the comparison and were therefore excluded from the analysis.

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### Table 1. The Color of Avulsed Teeth at Baseline and 12 Months and the Color Change That Took Place during This Period

<table>
<thead>
<tr>
<th>Color variables Randomization</th>
<th>No. of teeth</th>
<th>Mean at baseline (SD)</th>
<th>Mean at 12 months (SD)</th>
<th>Mean change (SD), 12 months–baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$ ++ Ledermix</td>
<td>10</td>
<td>58.9 (8.0)</td>
<td>53.8 (10.3)</td>
<td>-5.1 (5.8)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>12</td>
<td>56.2 (6.9)</td>
<td>58.1 (6.8)</td>
<td>1.9 (3.8)</td>
</tr>
<tr>
<td>$a^*$ (+) Ledermix</td>
<td>10</td>
<td>4.5 (3.6)</td>
<td>6.0 (6.4)</td>
<td>0.3 (5.5)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>12</td>
<td>3.6 (3.3)</td>
<td>4.7 (3.9)</td>
<td>0.3 (3.1)</td>
</tr>
<tr>
<td>$b^*$ ++ Ledermix</td>
<td>10</td>
<td>12.1 (5.1)</td>
<td>10.9 (6.8)</td>
<td>-1.2 (4.8)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>12</td>
<td>11.3 (3.0)</td>
<td>14.9 (2.8)</td>
<td>3.3 (2.4)</td>
</tr>
<tr>
<td>CIELAB $\Delta E$</td>
<td>10</td>
<td>8.12 (4.9)</td>
<td>8.12 (4.9)</td>
<td>0.0 (3.1)</td>
</tr>
</tbody>
</table>

All injured teeth were avulsed and replanted as part of multicenter RCT. For each variable ($L^*$, $a^*$, $b^*$ in the CIELAB space and CIELAB $\Delta E$) the data were separated into the 2 treatment groups the teeth were randomized to. CIELAB $\Delta E$ is a summary value that used data from each of the $L^*$, $a^*$, $b^*$ variables and reports the distance in a 5-dimensional model of color between the baseline and 12 months. This value only quantifies change of color but not the direction. The variables ($L^*$, $a^*$, $b^*$) report the individual component’s color (white–black, green–red, and yellow–blue).

SD, standard deviation.

++Significant difference between the Ledermix and Ultracal XS groups for $L^*$ by using a Mann-Whitney $U$ test and CIELAB $\Delta E$ by using an independent $t$ test.

(+)$Data are not normally distributed; therefore, the values for $a^*$ are given as a median and interquartile range.

### Table 2. The Change in Color Variables between an Avulsed Tooth Treated as Part of a Multicenter RCT and the Contralateral Nonavulsed Tooth

<table>
<thead>
<tr>
<th>Color variables for CIELAB space Randomization</th>
<th>No. of teeth</th>
<th>Avulsed tooth, mean (SD)</th>
<th>Nonavulsed contralateral tooth, mean (SD)</th>
<th>Difference between avulsed tooth and contralateral nonavulsed tooth (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$ ++ (+) Ledermix</td>
<td>7</td>
<td>62.4 (20.0)</td>
<td>54.4 (16.8)</td>
<td>-5.3 (6.8)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>5</td>
<td>67.9 (10.6)</td>
<td>67.1 (13.1)</td>
<td>0.3 (3.0)</td>
</tr>
<tr>
<td>$a^*$</td>
<td>7</td>
<td>6.4 (3.0)</td>
<td>5.4 (3.8)</td>
<td>1.0 (1.8)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>5</td>
<td>3.9 (1.4)</td>
<td>4.1 (1.6)</td>
<td>0.2 (0.3)</td>
</tr>
<tr>
<td>$b^*$</td>
<td>7</td>
<td>13.2 (7.3)</td>
<td>12.9 (5.4)</td>
<td>0.2 (3.8)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>5</td>
<td>13.9 (3.8)</td>
<td>11.5 (3.9)</td>
<td>2.4 (0.6)</td>
</tr>
<tr>
<td>CIELAB $\Delta E$ Ledermix</td>
<td>7</td>
<td>7.5 (4.2)</td>
<td>7.5 (4.2)</td>
<td>0.0 (3.1)</td>
</tr>
<tr>
<td>Ultracal XS</td>
<td>5</td>
<td>3.0 (1.3)</td>
<td>3.0 (1.3)</td>
<td>0.0 (3.1)</td>
</tr>
</tbody>
</table>

For central incisors (n = 10) this was compared with the nonavulsed central incisor; for canines (n = 2) this was compared with the contralateral canine. For each color variable ($L^*$, $a^*$, $b^*$ in the CIELAB space and CIELAB $\Delta E$) the data were separated into the treatment the teeth received. CIELAB $\Delta E$ is a summary value that used data from each of the $L^*$, $a^*$, $b^*$ variables and reports the distance between the avulsed and nonavulsed tooth in a 5-dimensional model of color. This variable only quantifies the change of color but not the direction. The variables ($L^*$, $a^*$, $b^*$) report the individual component’s color (white–black, green–red, and yellow–blue).

RCT, randomized controlled trial; SD, standard deviation.

++Significant difference between the Ledermix and Ultracal XS groups for $L^*$ by using a Mann-Whitney $U$ test and CIELAB $\Delta E$ by using an independent $t$ test.

(+)$Data are not normally distributed; therefore, the values for $L^*$ are given as a median and interquartile range.
investigated individual components of this medicament (8). For patients requesting treatment for their discolored teeth, the use of nonvital bleaching with carbamide peroxide was found to be effective.

The findings for Ultracal XS showed the medicament caused a change in color during the 12-month observation period of $L^* = 1.9$ (lighter), $a^* = 0.3$ (redder), and $b^* = 3.3$ (yellower). The increase in the yellowing ($b^*$ value) of teeth agreed with similar in vitro studies of mature and immature teeth (12, 13). The effect of increased lightening was not found either in the in vitro study or in Table 2, where the contralateral nonavulsed tooth was compared. This effect, although significant in comparison to Ledermix group, was more likely related to the significant discoloration seen in the Ledermix group. It is unproven why calcium hydroxide should cause yellowing of the tooth, but it might be related to the premature aging of the tooth similar to that seen with pulp canal obliteration.

There was no significant difference for $\Delta E$ between the 2 medicaments during the 12-month period. The reason for this was that both medicaments caused a change in color. $\Delta E$ quantifies the change in color but not the direction. It is only when the different components of color, $L^*$, $a^*$, $b^*$, are analyzed individually that the nature of the discoloration can be identified and the significant differences that are evident in the photographs become apparent.

The comparison to the nonavulsed contralateral tooth (Table 2) was a valid assessment because both patients and clinicians compare tooth color with contralateral teeth rather than by retaining a historical memory of its previous color. The $L^*$, $a^*$, $b^*$ variables in the nonavulsed contralateral tooth at the 12-month review were not significantly different between the 2 groups. Some of these teeth had suffered other traumatic injuries, and these injuries or subsequent healing modalities could have caused the color changes (28). If contralateral teeth had suffered discoloration, the randomization process appears to have allocated them equally to the 2 groups as determined by the lack of significant difference found. The mean or median CIELAB scores for the nonavulsed contralateral central incisor tooth ($L^* = 66.9$, $a^* = 3.8$, $b^* = 10.1$) were similar to those reported for maxillary incisor color (29). Where patients had avulsed both their central incisors, the usual reference point was lost to both patient and dentist alike. Consequently, where any discoloration was symmetrical, this was more likely to be acceptable to the patient. For this reason, children with avulsion of both central incisors were excluded from this part of the study.

For the Ledermix group there was an obvious mismatch between avulsed tooth and nonavulsed contralateral tooth. The size of $\Delta E$ change in color (7.5) was both perceivable and warranted further treatment if the values from previous studies of dentists’ perception are used (19, 20). The $\Delta E$ change in color for the Ultracal XS group was 3.0. The color change was consistent with earlier in vitro studies (12, 13), with Ultracal XS causing a yellowing of the tooth but with minimal lightening. The $\Delta E$ change was of the magnitude that would have been detected by dentists in one study (19) but not in the other (20), and neither study would have classified it as unacceptable.

Conclusions

Ledermix caused a significantly greater level of patient dissatisfaction with the color of avulsed and replanted teeth.

During a 12-month period Ledermix caused a significant gray-brown darkening of avulsed and replanted teeth.

During a 12-month period Ultracal XS caused a significant yellowing of the avulsed and replanted teeth.

In comparison to nonavulsed contralateral tooth, Ledermix caused a significant darkening of the tooth, whereas Ultracal XS caused yellowing, but this was not significant.

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The authors deny any conflicts of interest related to this study.

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