Abstract: There is some evidence of tooth discoloration after regenerative endodontic procedures. The aim of this review is to determine which materials used in regenerative procedures induce tooth discoloration. The authors searched six digital databases in February 2018: PubMed, Google Scholar, the Cochrane Library, Scopus, Web of Science, and Lilacs. As a result, 353 studies were found. Eleven studies that met the established criteria were selected. Analysis of the selected studies showed that most materials used in regenerative endodontic procedures have the potential to stain tooth tissues. To decrease tooth discoloration, sealing dentin with a dentin-bonding agent before the placement of a disinfectant paste and barrier materials should be considered. Only a small number of in vitro studies have been performed; therefore, further studies on this topic are warranted.

Keywords: calcium silicate-based cements; discoloration; regenerative endodontic procedures; revitalization.

Introduction

Regenerative endodontics, the newest branch of stomatology, studies the potential for the regeneration of damaged pulp as well as the creation and delivery of replacement pulp-dentin tissues (1). Potential regenerative endodontic techniques include root canal revascularization, pulp implants, three-dimensional cell printing, postnatal (adult) stem cell therapy, scaffold implants, injectable scaffolds, and gene therapy (1). The most common technique used is root canal revascularization, also called revitalization (2), which is used for the treatment of nonvital, infected, and immature permanent teeth (3).

Pulp necrosis of immature permanent teeth in children is commonly caused by injuries and caries (4) and often affects anterior teeth. The treatment of immature permanent teeth is particularly challenging for dentists, because these teeth are characterized by thin root walls and a wide apical opening. These attributes hinder correct root length measurement and airtight root filling, particularly in the apex. This increases the risk of tooth breaking. To date, cases of irreversible inflammation or pulp necrosis with incomplete root development are treated via the production of a mineralized barrier near the apex of the root during the process of apexification; however, this process does not induce tooth length growth, thickening of root walls, or the formation of the root apex (4,5), which occurs under physiological conditions, described...
as apexogenesis (6). Apexogenesis is possible only when the vital pulp or pulp-like tissue is present in the canal. Using regenerative endodontics, it is possible to recreate this process. According to the American Association of Endodontists (AAE), the regenerative process consists of removal of the infected pulp, disinfection of the root canal with disinfectant pastes, promotion of bleeding into the canal system by over-instrumenting, and placement of the barrier material over a blood clot (http://www.aae.org/regenerativeendo/, accessed on May 7, 2018). White mineral trioxide aggregate (WMTA) is recommended by AAE as a barrier material. However, mineral trioxide aggregate is associated with discoloration; therefore, bioceramics or tricalcium silicate cements (e.g., Biodentine, Septodont, Lancaster, PA, USA and EndoSequence BC RRM-Fast Set Putty, Brasseler USA [RRM]) should be considered in the esthetic zone. In some cases, promotion of bleeding is not possible, and AAE recommends the application of platelet-rich plasma, platelet-rich fibrin, or autologous fibrin matrix as an alternative therapy (http://www.aae.org/regenerativeendo/, accessed on May 7, 2018).

There are reports in the literature (7) that materials used in regenerative endodontic therapy have the potential to cause visible tooth discoloration, which may significantly and negatively impact the quality of life in children. Patients desire both successful treatment and an esthetic outcome, particularly for their front teeth; therefore, materials and procedures with the lowest possible staining potential must be carefully chosen.

Visual spectrophotometry is a widely and successfully used method of color evaluation in dentistry (8) and is currently considered the gold standard method. The delta E value defines the color difference between the final and initial values. The aim of this review is to evaluate the discoloration potential of factors and materials used in regenerative endodontic procedures based on published in vitro studies.

**Materials and Methods**

The main question aimed to answer in this paper was whether the materials and procedures used in regenerative endodontic procedures could cause discoloration of the hard tissues of the teeth. Publications were searched without a year limit in PubMed, Google Scholar, the Cochrane Library, Scopus, Lilacs, and Web of Science databases. The search phrases included “((regenerative endodontics OR regenerative procedure OR pulp therapy OR revascularization OR revitalization) AND (bioceramic OR biodentine OR mta OR mineral trioxide aggregate OR triple antibiotic paste OR tap OR blood OR prf OR prp) AND discolo*)”.

The final search was performed on February 18, 2018. Inclusion criteria comprised the following types of articles: in vitro studies, studies that used preparations placed into the tooth tissues, and those that described revitalization procedures.

**Results**

A total of 353 titles were retrieved by the query (71 in PubMed, 201 in Google Scholar, one in Lilacs, three in the Cochrane Library, 13 in Scopus, and 64 in Web of Science). Eleven studies were chosen according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards (Fig. 1). Table 1 shows information regarding the analyzed materials,
### Table 1 Data pertaining to analyzed studies

| Author and year | No. of teeth/ type of teeth | Irrigant(s) | Sample preparation method | Measurement method/ Measurement time | Material | Final considerations |
|-----------------|-----------------------------|------------|--------------------------|---------------------------------------|----------|----------------------|
| Lenherr et al. 2012 (9) | 210 Bovine teeth | 1% NaOCl 20% EDTA, tap water | Material placed in cuboid enamel-dentine blocks | Vita Easyshade Compact Before and after placement of material, 1 w, 1 m, 3 m, 6 m, 12 m | CG Blood ApexCal Ultracal XS TAP(m) GMTA GMTA + blood WMTA WMTA + blood PC PC + blood | Yes Specimens with TAP(m) were the most discolored. |
| Akcay et al. 2013 (10) | 70 Bovine teeth | 10 mL 5.25% NaOCl 10 mL 17% EDTA 10 mL distilled water | Canal filled with material | Spectro Shade Micro After placement of material, 1 d, 1 w, 2 w, 3 w | CG CH DAP TAP(m) TAP(d) TAP(a) TAP(c) | No Specimens with TAP(m) were the most discolored. |
| Feiman et al. 2013 (11) | 44 Human teeth | 6 mL 4% NaOCl 4 mL 15% EDTA 1 mL 4% NaOCl | Canal filled with material | Photoshop CS4 - Canon EOS TAP(m), after rinsing out | Saline Blood WMTA + saline WMTA + blood | No Specimens with blood were the most discolored. |
| Kirchhoff et al. 2014 (12) | 20 Human teeth | Distilled water 5 mL of 2.5% NaOCl | Materials placed into the pulp chamber | Vita Easyshade Advance 4.0 After placement of material, after 3 w of placement of TAP(m), after rinsing out TAP(m) | TAP(m) | Yes Discoloration was observed in all specimens. |
| Kohli et al. 2015 (13) | 80 Human teeth | 10 mL 3% NaOCl 10 mL 17% EDTA 10 mL distilled water | Canal filled with material | Ocean Optics Before placement of material, 1 w, 1 m, 2 m, 6 m | RRM RRMF BD WMTA GMTA TAP(m) CG | No Specimens with TAP(m) were the most discolored. |
| Detwiler et al. 2016 (14) | 330 Bovine teeth | 1% NaOCl 20% EDTA saline solution | Material placed in enamel-dentine block | Vita EasyShade Before and after placement of material 1 w, 1 m, 3 m, 6 m, 12 m | Empty Blood Ledermix MTA MM-MTA BD WMTA GMTA TAP(m) | No Specimens with blood were the most discolored. |
| Shokoubinejad et al. 2016 (15) | 104 Human teeth | 5.25% NaOCl 17% EDTA | Canal filled with material | Konika CS2000 Before placement of material, 1 d, 1 m, 6 m | PMTA + saline Ortho MTA + saline RRM + saline BD + saline PMTA + blood Ortho MTA + blood RRM + blood BD + blood | Yes All specimens with blood were more discolored than those with saline. |
| Santos et al. 2016 (16) | 50 Bovine teeth | 3 mL 1.5% NaOCl, 3 mL 17% EDTA distilled water | Canal filled with material | Vita Easyshade, VITA Classical A1-D4 Shade Guide Before and after placement of material 1 d, 3 d, 1 w, 2 w, 3 w, 5 m, 2 m, 3 m, 4 m after antibiotic paste placement 1 d, 3 d, 1 w, 2 w, 3 w, 5 m, 2 m after material GIC/WMTA placement | CG TAP(s) TAP(m) DAP CH TAP(m)/WMTA TAP(a)/WMTA DAP/WMTA CH WMTA TAP(m)/GIC TAP(a)/GIC DAP/GIC CH/GIC | No Specimens with WMTA were more discolored than those with GIC. |
| Akbulut et al. 2017 (17) | 42 Human teeth | 17% EDTA, 2.5% NaOCl | Canal filled with material | Vita Easyshade Advance Baseline, 3 w (TAP(c)) 4 w (PMTA; DB; MM-MTA + blood) | TAP(c) PMTA + blood BD + blood MM-MTA + blood | Yes Discoloration was observed in all specimens. |
irrigants, tooth type, sample preparation method, measurement time, measurement method, and discoloration (8-18). Figure 2 presents delta E values from studies that included its value in results (8,9,12,14,16,17); however, not all studies included numeric delta E values. A statistical analysis could not be performed because of different parameters used by reviewed studies. The risk of bias was evaluated according to the modified Methodological Index for Non-randomized Studies (MINORS) scale (Fig. 3). All the analyzed studies presented a low risk of bias.

Discussion

This review identified 11 in vitro studies that have reported tooth discoloration induced by materials used in regenerative endodontic techniques. Notably, most of

| Author and year | No. of teeth/ type of teeth | Irrigant(s) | Sample preparation method | Measurement method/ Measurement time | Material | Discoloration |
|----------------|-----------------------------|------------|--------------------------|--------------------------------------|----------|--------------|
| Shokouhinejad et al. 2017 (18) | 96 Bovine teeth | 20 mL 1.5% NaOCl, 20 mL 17% EDTA | Canal filled with material. In some groups, the access cavity walls were sealed with DBA before placement of the material | Vita Easyshade Before placement of materials, 4 w after placement of antibiotic paste After placement of barrier material After filling of the access cavity 4 m after filling of access cavity | TAP(m), PMTA + blood TAP(m), RetroMTA + blood TAP(m), BD + blood TAP(m), PMTA + blood/DBA TAP(m), OrthoMTA + blood/DBA TAP(m), RetoMTA + blood/DBA TAP(m), BD + blood/DBA | Yes | Specimens without DBA discolored more than those with DBA. |
| Iriboz et al. 2017 (8) | 85 Human teeth | 2 mL 5.25% NaOCl, 10 mL 5.25% NaOCl, 10 mL 17% EDTA, 10 mL distilled water | Canal filled with material | Vita Easyshade 0 d, 1 w, 2 w, 3 w, 4 w | TAP(m) MP CH | Yes | Discoloration was observed in specimens with TAP(m) and MP. |

CG: control group; CH: calcium hydroxide; DAP: double antibiotic paste; TAP(m): triple antibiotic paste with minocycline; TAP(d): triple antibiotic paste with doxycycline; TAP(a): triple antibiotic paste with amoxicillin; TAP(cf): triple antibiotic paste with cefuroxime; BD: Biodentine; RRM: EndoSequence Root Repair Material putty; RRMF: EndoSequence Root Repair Material fast set paste; WMTA: white MTA; GMTA: grey MTA; MP: minocycline paste; PMTA: Pro Root MTA; PC: Portland Cement; PC Bi2O3: Portland cement with added bismuth oxide; PC ZrO: Portland cement with added zirconium oxide; GIC: glass ionomer cement; MTA: mineral trioxide aggregate; DBA: dentin-bonding agent; d: day; m: month; w: week.

Fig. 2 Delta E values defining the color changes of materials in reviewed studies. The numbers in parentheses are reference citations.

CG: control group; CH: calcium hydroxide; DAP: double antibiotic paste; TAP(m): triple antibiotic paste with minocycline; TAP(a): triple antibiotic paste with amoxicillin; TAP(cf): triple antibiotic paste with cefuroxime; BD: Biodentine; RRM: EndoSequence Root Repair Material putty; RRMF: EndoSequence Root Repair Material fast set paste; WMTA: white MTA; GMTA: grey MTA; MP: minocycline paste; PMTA: Pro Root MTA; PC: Portland Cement; PC Bi2O3: Portland cement with added bismuth oxide; PC ZrO: Portland cement with added zirconium oxide; GIC: glass ionomer cement; MTA: mineral trioxide aggregate; DBA: dentin-bonding agent; d: day; m: month; w: week.
the studies analyzed in this review used different sample preparation procedures. For instance, Lenherr et al. (9) and Dettwiler et al. (14) placed the studied materials in cuboid enamel-dentine blocks, whereas Kirchhoff et al. (12) used pulp chambers. However, some researchers studied materials in the root canal (8,10,13,16,18). Different methodologies in some instances led to different results.

A vast amount of research has measured tooth discoloration using a spectrophotometer and evaluated discoloration with the CIELAB color space technique (defined by the International Commission on Illumination (CIE)) (9,10,12-14,16-18). Felman et al. (11) obtained images with a digital camera and used Adobe Photoshop CS4 to perform shade analysis with a CIELAB color space. Schokouhinejad et al. (15) measured discoloration with a spectroradiometer. Analysis of all the retrieved data showed that tooth discoloration could occur at any stage of treatment, such as after the use of a disinfectant paste, promotion of bleeding, or placement of barrier materials.

Disinfectant pastes are intracanal medications used to disinfect the canal during pulp revitalization. Seven papers studying triple antibiotic paste (TAP) with minocycline were found (8-10,12,13,16,18). Four of them compared TAP to different materials and found it to be the most potent in terms of staining ability (9,10,13,16), with minocycline having the greatest staining potential of the other three antibiotics (19). One of the studies found that discoloration was permanent despite rinsing out the paste (12). Berkhoff et al. (20) noted that regardless of the irrigation techniques used, around 88% of TAP with minocycline was found in the root canal system. TAP with minocycline was circumferentially presented up to 350 µm within the dentin (20). Minocycline is a semi-synthetic tetracycline derivative which staining potential was likely related to its chelation of calcium ions (19) and incorporation into the tooth tissue (12) as well as the formation of an oxidation product (21). TAP with minocycline caused the greatest tooth discoloration (9,10,13,16), even when compared to specimens containing only blood (9). Kirchhoff et al. (12) studied the ability of sodium hypochlorite (NaOCl) to bleach teeth by rinsing out TAP with minocycline from the canal. They found that NaOCl was not enough to cause bleaching. According to the AAE, minocycline could be omitted from or replaced with other antibiotics in TAP, such as cefaclor, clindamycin and amoxicillin (http://www.aae.org/regenerativeendo/, accessed on May 7, 2018). The staining potential of TAP with amoxicillin was evaluated by two studies, which showed a discrepancy in their outcomes (10,16). Ackay et al. (10) observed the staining potential of TAP with amoxicillin; this result was in contradiction with findings by Santos et al. (16). There was also a difference in methodologies of these studies. Ackay et al. (10) rinsed root canals with 10 mL of 5.25% NaOCl, and Santos et al. (16) rinsed them with 3 mL 1.5% NaOCl before placement of the antibiotic paste. In addition, Ackay et al. (10) placed TAP with amoxicillin through the apical aspect of teeth and did not include information about the concentration of the antibiotic paste. Alternatively, Santos et al. (16) used 0.1 mg/mL TAP with amoxicillin that was placed into the root canal through endodontic access. Some authors have reported that TAP with cefaclor induced tooth discoloration (10,17). Ackay et al. (10) and Santos et al. (16) claimed that double antibiotic paste placed in the root canal was not the cause of tooth discoloration; this was in contrast with findings by Dettwiler et al. (14), who used enamel-dentin blocks as samples, which did not fully correspond to real teeth. Calcium hydroxide exhibited no staining potential in the reviewed studies (8-10,16), but it was noted that ApexCal (Ivoclar Vivadent, Schaan, Liechtenstein), which is a calcium hydroxide-based product with bismuth carbonate, showed the ability to discolor teeth (9). Bismuth ions, which are derivatives of bismuth carbonate, could be responsible for ApexCal’s staining potential.

The next stage of revitalization was the promotion of bleeding into the root canal by over-instrumenting the apex, followed by the placement of a coronal barrier material over the blood clot. A blood clot formed inside the canal could affect the color of the teeth. This discoloration was most likely caused by the distribu-
tion of blood products, such as hemoglobin, hematin, and erythrocytes, which penetrated the dentinal tubules (22). In a histochemical study, Martin et al. (22) noted that most discoloration could occur in the dentine layer near the pulp chamber and its intensity is decreasing towards the outer tooth surface. Blood had high staining potential which occurred with (9,11,15,17) and without contact with barrier materials (9,11,14,15). Lenherr et al. (9) stated that after blood contamination of Portland Cement (PC; Medcem GmbH, Weinfelden, Switzerland) and WMTA (Angelus, Londrina, PR, Brazil), increased discoloration was observed. Shokouhinejad et al. (15) compared the effect of blood contamination of barrier materials on tooth color and concluded that all materials that were in contact with the blood presented higher staining potential than materials that were in contact with saline. The materials included Biodentine (Septodont, Saint Maur des Fosses, France); ProRoot MTA (Dentsply, Tulsa, OK, USA); Ortho MTA (BioMTA, Seoul, Republic of Korea); and RRM. Only one study examined the influence of plasma and platelet concentrate on tooth discoloration. Marin et al. (22) reported that teeth with a pulp chamber filled with plasma and platelet concentrates did not demonstrate color changes compared to teeth filled with sterile saline.

Many authors noted that materials used in regenerative procedures as barrier materials caused tooth discoloration. Discoloration was largely related to the composition of materials used, such as bismuth, iron, aluminum, and magnesium oxides (15,23). The mechanism of tooth discoloration caused by bismuth oxide was based on the oxidation of bismuth oxide into bismuth carbonate. This reaction occurred when bismuth was in contact with carbon dioxide from the air. Reduction of bismuth oxide to bismuth metal could also cause discoloration (24). Marciano et al. (24) reported that when in contact with collagen, bismuth oxide changed to a black sediment. Camilleri observed discoloration of WMTA after immersing material in NaOCl (25). WMTA contained bismuth oxide, which was discolored after contact with NaOCl. WMTA in contact with ethylenediaminetetraacetic acid (EDTA) showed significant discoloration compared to samples of material immersed in normal saline and samples left in dry condition. This was in disagreement to situations when Biodentine was used and color stability was observed (26). Grey MTA (GMTA; Angelus, Londrina, PR, Brazil) (9,13) and WMTA (9,13) cements also caused discoloration, which was probably induced by their radiopacifying component, bismuth oxide.

Many manufacturers modified the composition of materials by reducing the content of bismuth oxide or replacing it with zirconium oxide, which is also used as a contrasting material (27). Some studies report that zirconium oxide does not have a high staining potential (14,23,28). Dettwilet et al. (14) evaluated PC, PC with bismuth oxide, and PC with zirconium oxide. In this study, discoloration was observed in PC with bismuth oxide, but not in MM-MTA (Micro Mega, Besancon Cedex, France) and Ledermix MTA (Riemser Pharma GmbH, Greifswald-Insel Riems, Germany), although these two products contained bismuth oxide. The authors did not provide an explanation of this phenomenon. They noted that the occurrence and concentration of bismuth oxide in calcium silicate cements was not a reliable indicator of the possibility of discoloration. Marciano et al. (29) further investigated whether there was a correlation between the concentration of bismuth oxide and color change in calcium silicate cements. Authors noticed that despite increasing radiopacity, bismuth oxide did not influence intensity of discoloration (29). It was probable that composition of the calcium silicate cements could affect tooth discoloration. Biodentine (13,14), RRM, and EndoSequence Root Repair Material fast set paste (RRMF; Brasseler USA, Savannah, GA, USA) (13), which contain zirconium oxide, did not produce discoloration in separate samples. Discoloration was induced with ProRoot MTA (15), OrthoMTA (15), RRM (15), Biodentine (15), and WMTA (11) evaluated in samples with blood and saline. Blood contamination had a strong impact on tooth color. WMTA and GMTA caused visible tooth discoloration after 6 months, as opposed to Biodentine, RRM and RRMF (13). The difference was related to the replacement of bismuth oxide with zirconium oxide in Biodentine, RRM, and RRMF. Shokouhinejad et al. (15) reported that OrthoMTA in contact with saline presented greater tooth discoloration after 6 months than Biodentine and RRM did. WMTA also had staining potential, but it was greater when the material was contaminated with blood (11). Uptake of blood components in porosities of barrier materials could be a potential mechanism of color change (9).

AAE recommends using a dentin-bonding agent on dentin in the pulp chamber and using TAP below the cementoenamel junction to mitigate tooth discoloration. Shokouhinejad et al. (18) evaluated the impact of the use of pulp chamber dentin insulation with a dentin-bonding agent before placing TAP with minocycline into a root canal and before placing blood and barrier materials on staining potential (18). The authors stated that this procedure decreased tooth discoloration but did not eliminate the problem (18,19).
Conflicts of interest

The authors declare no conflict of interest related to this study.

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