Review Article

REGENERATIVE ENDODONTICS: A REVIEW ON CLINICAL PROTOCOLS AND SUBSEQUENT CORONAL DISCOLORATION

Farbod M, [1] *Bolhari B* [2]

1. *DDS, Post Graduate Student, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, IRAN.*
2. *DDS, MSc., Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, IRAN.*

Abstract

Regenerative endodontic therapy is known as treatment of teeth with infected root canal systems by regenerative endodontic protocols. Despite the great advantages, discoloration of the crown of the tooth is a reported unfavorable outcome associated with regenerative endodontic therapy. Discoloration is a clinical problem because discoloration of teeth in particular has been related to a negative impact on the quality of life in patients. Thus, the purpose of this study was to determine the crown discoloration following regenerative endodontic treatment using the PubMed and Medline database English literature by the terms "coronal discoloration", "Regenerative endodontic", "Scaffolds", "Calcium silicate", "Mineral trioxide aggregate", "Platelet-rich fibrin". In conclusion the calcium hydroxide or the double antibiotic paste of metronidazole and ciprofloxacin may limit discoloration. Mineral trioxide aggregate was also associated with discoloration. It has been suggested that one possible way to avoid discoloration is to seal the dentinal walls of the access cavity with a dentin bonding agent. The most concern on regenerative endodontic treatment is tooth discoloration induced by endodontic materials. For this reason, the selection of material is crucial to use in the esthetic zone. Because discoloration is a patient oriented outcome, further research should document and identify the incidence of discoloration in regenerative endodontic treatment.

Key words: Calcium silicate, Coronal discoloration, Regenerative endodontic, Scaffolds.

Introduction

Regenerative endodontic protocols usually contain disinfection of the root canal and the introduction of a blood clot, stem or progenitor cells into the root canal space and restoration with microorganism impregnable material.[1] In the regenerative endodontics damaged structures and cells in the pulp–dentin complex replace with live viable tissue, which restore normal physiologic functions. Regeneration rather than replacement of tissues with an artificial substitute is an emerging and exciting field of medicine.[3]

Tooth discoloration creates a range of aesthetic problems, and considerable amounts of time and money are invested in attempts to improve the appearance of discolored teeth. Discoloration is a more significant factor for many people in achieving an aesthetic smile than restoring their normal alignment within the arch.[3] Therefore, it is important for dental professionals to have a thorough knowledge and understanding of the aetiology and clinical features of tooth discoloration to select the most appropriate treatment for each case. Any changes of tooth structure are likely to cause an alteration in outward appearance of the tooth caused by changes of light transmitting and reflecting properties.[4] Some discolorations are located on the outer surface of the tooth structure, others are caused by stain taken up by the enamel or dentin, and some occur during tooth development and result in an alteration of the light transmitting properties of the tooth structures.[5] Tooth discoloration induced by endodontic materials is usual concern for treatment quality. Material penetration into dentinal tubules has been proposed as the main cause of discoloration over time.[6] Tooth discoloration also happens via the transmission of material discoloration by hard tissues. For this reason, the selection of material is crucial to use in the esthetic zone.[6] Many studies have shown that discoloration is a significant negative outcome following regenerative endodontic procedures. This is of particular concern for traumatized anterior teeth, as appearance and pleasing aesthetics are patient-centered outcomes.[1] A combination of ciprofloxacin, metronidazole and minocycline is suggested for bacterial disinfection. Although its efficacy for bacterial disinfection is superior to traditional intracanal medicaments; discoloration caused by the combination is well documented. Application of the antibiotics as well as coronal barriers are the main reason for discoloration.[1] Lenherr *et al*[7] reported that the calcium hydroxide dressings did not show any discoloration or differ from the negative controls at any time point. Crown discoloration related to endodontic filling materials is associated with the material time to contact the tooth structure, as well as the potential chromogenic materials used in the treatment. Alternative materials including zirconium oxide and tantalum oxide are used in recent years to replace bismuth oxide and the contents of the heavy metals as well as color changes in calcium silicate-based materials. Therefore, the purpose of this literature review was to investigate findings and reports on the crown discoloration following regenerative endodontic treatment. Based on the evidence currently available, it is not possible to determine the incidence of tooth discoloration after regenerative endodontic treatment. If treatment decision making and informed consent are based on published literature, the evidence should be as complete as possible. Adverse effects, including crown/root discoloration, should be reported in order to give a broader view to clinicians and patients. For example, it is reasonable to expect cases in which the regenerative procedure does not succeed but discoloration still occurs although these cases are unlikely to be reported because of publication bias.

Material and Methods

The literature search for this review was peer-reviewed articles with following key-words: coronal discoloration × Regenerative endodontic × Scaffolds × Calcium silicate × Mineral trioxide aggregate × Platelet-rich fibrin. Among

them, the papers were fit the criteria selected and available full-text articles read. Related articles were also scrutinized. Hand search was also driven. The search was carried out using Biological Abstracts, Chemical Abstracts, and the data bank of the PubMed and Medline database updated to 2017. The references found in the search were then studied in detail.

Regenerative endodontic procedures

Regenerative endodontics is an exciting and rapidly developing field in the treatment of immature teeth with infected root canals and arrested root development. REPs have been described as a paradigm shift in the management of these teeth and can result in continued root lengthening, wall thickening and apical closure.[8] Regenerative endodontics has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex" (13). Dental stem cells capable of differentiating into odontoblast-like cells and regenerative endodontic therapy can promote thickening of the canal walls and continued root development of immature permanent teeth with necrotic pulps. The biological concept of regenerative endodontics involves the triad of stem cells, scaffold platforms and signaling molecules.[2] The clinical considerations for regenerative endodontic protocols are disinfection of the root canal system; provision of a scaffold in the form of a blood clot that forms after laceration of the periapical tissue to induce bleeding and introduce mesenchymal stem cells within the root canal; and an adequate coronal seal to prevent reinfection.[9] The current recommended clinical protocol for REPs is described by the American Association of Endodontists.[10]

Intracanal medicaments

The success of revascularization/revitalization therapy depends on efficient disinfection of the root canal system. The earliest case reports describing the disinfection protocol in the treatment with regenerative endodontic procedures involved medicating the canals with a combination of antibiotics.[8] The traditional triple antibiotic paste (TAP) consisting of ciprofloxacin, metronidazole, and minocycline has good antimicrobial properties in infected root canals.[1] Despite these positive features, numerous case reports have shown that minocycline causes visible crown discoloration.[11] Although main reason for discoloration after regenerative endodontic treatments is minocycline, several studies revealed that gray MTA and white MTA can cause discoloration after treatment. A practical way to prevent discoloration is replacing minocycline with an antibiotic that does not stain teeth. It is reported a successful regenerative endodontic treatment of a maxillary central incisor by using cefaclor instead of minocycline in the antibiotic mixture. Some efforts have been made to omit intracanal medication from the treatment procedure. It is presented a successful single-visit technique of regenerative endodontic treatment. The technique used in this study consisted of irrigation of the coronal portion of the root canal space with NaOCl and chlorhexidine gluconate and then MTA placement without bleeding induction.[12]

Although other earlier reports advocated the use of calcium hydroxide exclusively the vast majority of published cases seem to have employed a combination of antibiotics as the preferred intracanal medicament.[13] However, these antibiotic pastes have been shown to be cytotoxic to the survival of SCAP in concentrations equal to or higher than 1 mg/mL in in vitro studies. In contrast, the disinfection of root canal space with calcium hydroxide promoted the proliferation of SCAP.[14] Calcium hydroxide also increased the release of growth factors from dentin, whereas antibiotic pastes negatively influenced growth factor release after the use of EDTA.[15] The AAE clinical considerations for a regenerative endodontic procedure advocate the use of either a combination of antibiotic paste or calcium hydroxide paste.[10] A retrospective radiographic study employing a quantitative analysis of teeth treated with REPs showed that teeth medicated with calcium hydroxide had a significantly greater increase in root length than the teeth medicated with the combination of antibiotic paste. Importantly, teeth treated with the combination of antibiotic paste had significantly greater increases in root canal wall thickness. The placement of calcium hydroxide within the canal appeared to favourably affect the outcome of the treatment. When calcium hydroxide was restricted to the coronal half of the root canal, as demonstrated radiographically, the median increase in the dentinal wall thickness was 53.8%. This contrasted with just a 3.3% increase when calcium hydroxide was present in the apical half of the root canal. The percentage change in root length was not affected in either of these different clinical protocols.[16] The results of a cohort study of 12 teeth medicated with a triple antibiotic paste showed apical closure in 66.7% of cases, increased root wall thickness in 41.7%, and increased root length in 41.7% of cases. In comparison, 11 teeth medicated with calcium hydroxide showed apical closure in 54.5% of cases, increased root canal thickness in 45.4%, and increased root length in just 27.3% of cases.[16] These findings were considered comparable outcomes, and hence, the application of either medicament is supported for use in the regenerative endodontic procedures.[16] Histologic evidence of successful treatment in this study demonstrated that the introduced method of root canal disinfection is a promising protocol for immature necrotic teeth, and the use of triple antibiotic paste may not be necessary. Studies introduced novel methods to shorten the treatment period and also prevent tooth discoloration by omitting the intracanal medication process. In a study on spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization, Akcay *et al*. revealed control, calcium hydroxide, and double antibiotic paste (DAP) groups showed no color changes exceeding the perceptibility threshold at all-time points. The results indicated that all antibiotic pastes, except DAP, induced crown discoloration.

Coronal barriers

Mineral trioxide aggregate

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early1990s.[17] MTA was first described in the dental scientific literature in 1993 and was given approval for endodontic use by the U.S. Food and Drug Administration in 1998.[18] As it will soon follow, MTA materials are derived from a Portland cement parent compound; it is interesting that no information has been published regarding to any investigations that led to the precise delineation of the present MTA materials. Because existing materials did not have ideal characteristics, MTA was developed and recommended for pulp capping, pulpotomy, apical barrier formation in teeth with necrotic pulps and open apexes, repair of root perforations, root-end filling, and root canal filling. The MTA materials are a mixture of a refined Portland cement and bismuth oxide, and are reported to contain trace amounts of SiO_2, CaO, MgO, K_2SO_4, and Na_2SO_4.[19] The major component, Portland cement, is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalcium aluminoferrite.[20] Gypsum is an important determinant of setting time, as is tetracalcium aluminoferrate, although to a lesser extent. MTA products may contain approximately half the gypsum content of Portland cement, as well as smaller amounts of aluminum species, which provides a longer working time than Portland cement. The MTA materials have been reported to solidify similar to other mineral cements, in which the anhydrous material dissolves, followed by the crystallization of hydrates in an interlocking mass.[21] The basic framework of the hydrated mass is formed by the interlocking of cubic and needle-like crystals in which the needle-like crystals form in sharply delineated thick bundles that fill the inter-grain space between the cubic crystals.[22] The effect of mixing MTA powder with different liquids and additives has shown that the choice of preparation liquid can have an effect on setting time and compressive strength.[23] Up to 2002, only one MTA material consisting of gray colored powder was available, and in that year white mineral trioxide aggregate, white MTA, was introduced as ProRoot MTA (Dentsply Endodontics, Tulsa, OK, USA) to address esthetic concerns.[19] After that time, two forms of MTA materials were categorized: the traditional gray MTA and WMTA. Scanning electron microscopy (SEM) and electron probe microanalysis characterized the differences between GMTA and white MTA and found that the major difference between gray MTA and white MTA is in the concentrations of Al_2O_3, MgO and FeO.[24] The mechanisms how white MTA influences on coronal tooth discoloration and those by which blood exacerbates this discoloration are not fully elicited. The possible mechanism is the oxidation and incorporation of the remaining iron content within the white MTA powder into the calcium aluminoferrite phase of the set white MTA cement.[25] Even though white MTA has 9% of the iron oxide of gray MTA, this quantity may be adequate for causing discoloration.[25] White MTA also interacts with erythrocytes. Discoloration of traumatized teeth results from the hemolysis of erythrocytes and the accumulation of hemoglobin and hematin molecules within dentin tubules.[25] The slow hydrating process of white MTA may permit the absorption and subsequent hemolysis of erythrocytes from the adjacent pulpal tissue, thus resulting in both material and subsequent tooth discoloration.[25] The descriptions of various bioactive molecules including growth factors lead to exciting alternative treatments of dentin-pulp complex. The usage of growth factors alone in regenerative treatment approaches tried to be developed imitating the physiological events of the body has been questioned.[26] Newly formed tissues in the canals could extend to the surface of MTA in some cases or occupy half of the canal space after 3 months. The growth of the tissue into the canal seemed not limited by the blood supply with an apical opening of 0.8 mm in diameter. When tissues engineered in the laboratory are implanted into the human body, only cells within 100–200 mm from the nearest capillary can attain sufficient diffusion of nutrients to survive. Thus, it was suggested that a voluminous tissue be prevascularized for achieving immediate and sufficient blood supply after implantation.[27] MTA is a good material for pulp capping and apexification, which can induce dental pulp cell differentiation and the secretion of mineralized tissue.[0] Cells in new vital tissues in the apical canal were more immature with larger and deeply stained nuclei. These cells pertain more potential for multilineage differentiation. Cementum-like tissue was along the internal root canal walls. In some cases, cementum-like tissue inside the root canal was connected with root surface cementum. The source of stem cells responsible for bone-like and cementum-like tissues is not clear, possibly from the periapical tissues.[27] Discoloration after treatment of teeth that were treated with calcium hydroxide might be related to presence of MTA in cervical portion of the root canal space. A recent report on pulp capping in anterior teeth revealed that presence of white MTA in the crown can cause considerable discoloration.[28] In clinical and radiographic follow-ups, both teeth were functional, periapical lesions were healed, and the apices formed. However, the roots were not developed. After 6 years, because of moderate discoloration and caries, teeth received root canal therapy and were permanently restored with casting dowel core and full crown restorations.[28] A disadvantage of the MTA is discoloration of the coronal dentin when placed in the canal.[6] McTigue *et al* [29] reported that 14 teeth discolored. The first 10 cases were treated with TAP including minocycline, which was then discontinued and substituted with clindamycin because of concerns with discoloration. Gray MTA, which has been linked with tooth discoloration was used in the first 12 cases, and this was then substituted with white MTA because of concerns with discoloration. Although 7 of the discolored teeth occurred when minocycline and gray MTA were used, 7 teeth also discolored when neither minocycline nor gray MTA was used.[11] These authors noted that white MTA has been implicated in tooth discoloration. Spectrophotometric analysis of coronal discoloration induced by gray and white

MTA, found that both materials discolored teeth. However, the effect was more marked with gray MTA.

Calcium silicate-based materials

Calcium silicate based materials has several advantages such as adherence to root dentin, forming a crystalline bond in a biochemical process termed biomineralisation.[30] The biomineralisation ability of Biodentine initiates calcium and silicate uptake by the dentin, which in turn would cause chemical and structural modification of dentin that may result in higher acid resistance and physical strength.[30] Calcium silicate-based cements adhere to root dentin, forming a crystalline bond in a biochemical process termed biomineralisation.[12] Hence, the use of Biodentine as an obturation material may eventually improve the resistance of the endodontically treated immature teeth against fracture. From an earlier in vitro study, it could be concluded that mineral trioxide aggregate significantly strengthened immature root canals.[10] Moreover, Biodentine exhibits greater compressive strength in comparison to other tricalcium silicate cements, which is attributed to the low water/cement ratio made possible by the water soluble polymer in the liquid. The physical properties of Biodentine such as flexural strength, elastic modulus and Vickers hardness are similar to dentin.[20] Progressive discoloration of the tooth crown is a potential aesthetic complication after endodontic treatment of immature anterior teeth. Discoloration is either a result of materials ingressing into dentinal tubules or by material remnants in the pulp chamber, which get darker over time and is transmitted through the hard tissues.[7] In the present report, no discoloration was observed in any of the three cases after using Biodentine. This is consistent with the literature as Biodentine exhibited color stability independent of oxygen and light irradiation unlike other tricalcium silicate cements such as mineral trioxide aggregate.[11] Biodentine ™, as a suitable material, has been suggested for the purpose of dentin-pulp complex regeneration in the clinical setting.[13] In a previous study Biodentine ™ was used in the revascularization process and reported resolution of the associated periapical pathology in a mandibular incisor of a 15 year-old patient.[30] In the afore-mentioned study, the tooth was irrigated with 6% NaOCl and the triple antibiotic paste was applied as the intracanal medicament with no instrumentation. Biodentine ™ was then applied to achieve a coronal seal. The tooth was then permanently restored with bonded resin. The lesion resolved after 18 months. The authors stated that Biodentine ™ was suitable for maintaining the vitality of dental pulp stem cells and creating a suitable environment for revascularization of dental pulp and consequent completion of root maturation.[13] In a study on evaluation and comparison of occurrence of tooth discoloration after the application of various calcium silicate-based cements revealed that there was no significant difference between tooth discolorations with Biodentine (Septodont, Saint Maur des Fosses, France), OrthoMTA (BioMTA, Seoul, Korea), and EndoSequence Root Repair Material (ERRM; Brasseler, Savannah, GA) in the presence of blood. However, in the absence of blood, Biodentine and ERRM exhibited less tooth discoloration than OrthoMTA. Biodentine was used as a double seal calcium silicate material, which has an extensive range of application with shorter setting time than MTA.[22] Biodentine accelerates immortalized murine pulp cell differentiation into odontoblast cells and biomineralization.[22] Biodentine modulates transforming growth factor β-1 secretion by pulp cells and causes minor discoloration compared with mineral trioxide aggregate.[22]

Dentin bonding agents

One of the methods to decrease discoloration is using dentin bonding agents. Kim *et al*[13] examined the performance of this prevention technique for tooth discoloration. In this study teeth treated with dentin bonding were evaluated with naked eye and then with colorimeter. In the eye assessment teeth did not have any change in color, but in the colorimeter assessment they had. They concluded that using dentin bonding agents before placement of the triple antibiotic paste might not completely prevent tooth discoloration. Interestingly, a recent research on an approach to eliminate tooth discoloration revealed that sealing the dentinal tubules of the pulp chamber with a bonding agent is a viable alternative although still not completely efficacious.[2] The dentin bonding agent can only decrease the intensity of the discoloration.[13] One possible way to minimize discoloration is to seal the dentinal walls of the access cavity with dentin bonding agent.[11] Sealing the dentinal tubules of the chamber inhibits the undesirable crown discoloration produced through tri-antibiotic medication whilst maintaining the revascularization potential of the pulp.[8] The administration of the dentin bonding agent before filling the pulp cavity with MTA prevented the MTA component from penetrating into the dentinal tubules which decreased discoloration. Applying a dentin bonding agent before MTA placement can minimize tooth discoloration. Keskin *et al*[6] reported that discoloration induced by calcium silicate-based materials diminished by the administration of a double layer of dentin bonding agent in the access cavity or via treating with internal bleaching. The application of dentin bonding agent, to seal dentinal tubules before placement of the white and grey MTA, prohibited dental discoloration.[15] The application of a dentin bonding agent requires time and accuracy to attempt to prevent WMTA discoloration.[15] In a study on prevention of coronal discoloration induced by regenerative endodontic treatment in an ex vivo model revealed sealing the pulp chamber walls before insertion of TAP decreased coronal discoloration following REP using different endodontic cements but did not prevent it. Discoloration of teeth undergoing REPs is an unfavorable outcome. Considering the significant contribution of TAP containing minocycline to the coronal tooth discoloration even after sealing the pulp chamber walls, the revision of current guidelines in relation to the use of TAP with minocycline might need to be revised.

Scaffolding Matrix

Platelet-rich fibrin

3D scaffolds are essential parts of the tissue engineering triad. An ideal scaffold can significantly facilitate attachment, migration and proliferation of stem cells, and 3-D spatial organization of stem cells as well as infiltration of host cells. While the current REP protocol assumes an endogenous scaffold from fibrin of clotted blood, other autologous sources such as PRP and platelet-rich fibrin (PRF) have also demonstrated comparable outcomes in animal as well as human clinical reports. Other exogenous synthetic matrices include injectable collagen scaffold and chitosan nanoparticles as well as products of dermal fillers such as Restylane (QMed, Uppsala, Sweden) are under investigation.[12] In addition to providing a structural matrix for stem cells, functional modifications to scaffolds can uniquely add to their utility in a REP. For example, one technical limitation of REPs is to predictably evoke bleeding from periapical tissues. In cases where adequate bleeding to the CEJ cannot be evoked scaffolding material can be supplemented with chemoattractants that can facilitate stem cell migration. To this end, a recent in vitro study evaluated several chemoattractants and found Granulocyte-Colony Stimulating Factor (G-CSF) and Fibroblast-Growth Factor-2 (FGF-2) to have considerable chemoattractive properties for SCAP (17). In transfusion medicine, platelet concentrates were originally used for the treatment and prevention of hemorrhage due to severe thrombopenia, which is often caused by medullar aplasia, acute leukaemia or significant blood loss during long-lasting surgery. The standard platelet concentrate for transfusion has been named PRP and classically contains 0.5×10^{11} platelets per unit. The use of blood-derived products to seal wounds and stimulate healing started with the use of fibrin glues, which were first described 40 years ago and are constituted of concentrated fibrinogen (polymerization induced by thrombin and calcium). Platelet-rich fibrin described is a second-generation platelet concentrate which allows one to obtain fibrin membranes enriched with platelets and growth factors, after starting from an anticoagulant-free blood harvest without any artificial biochemical modification. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest (6) and shows a complex architecture as a healing matrix, including mechanical properties no other platelet concentrate offers. The PRF can be considered as a natural fibrin-based biomaterial favourable to the development of a micro vascularization and able to guide cell migration into wound area. Its chief advantages include ease of preparation and lack of biochemical handling of blood, which makes this preparation strictly autologous.[10]

To increase the success rate, a critical need exists to develop new biologically based therapeutics that reduce pulp inflammation and promote the formation of dentin pulp tissues.[13] However, it was shown that the newly grown tissues into the root canal space have little similarity to normal pulp tissue but with more resemblance to cementum, periodontal ligament, or bone. The cause of this outcome is possibly related to the lack of stem cells derived from remaining vital pulp and apical papilla, which are destroyed by severe endodontic infection. Stem cells responsible for newly regenerated tissues might be derived from several other sources, including systemic blood or local tissue such as bone.[11] Furthermore, whether these newly formed tissues can function like normal pulp and stabilize the tooth without giving rise to further infection or canal obliteration still remains unknown. They also found that transplantation of unfractionated total pulp cells into root canal showed less tissue formation followed by evidence of mineralization on day 90 compared with transplantation of $CD10^{5+}$ pulp cells and stromal cell–derived factor-1. Although DPSCs are the most direct cell source in dental pulp regeneration, a number of other cell sources including SCAPs and bone marrow mesenchymal stem cells may also contribute to dental pulp regeneration. This may be another reason why the transplantation of autologous DPSCs alone did not help dental pulp regeneration. Further studies are needed to identify the cell sources of the tissues formed in the canal space (i.e., from periapical tissues or from the transplanted DPSCs). Growth factors and a suitable scaffold are also essential considerations in tissue regeneration. PRP contains several growth factors including transforming growth factor β1, platelet-derived growth factor, fibroblast growth factor, vascular endothelial growth factor, and epidermal growth factor that support cell growth.[10]

Conclusion

In conclusion 3 factors affect discoloration including intracanal medication, coronal barrier and using dentin bonding agent. It has been suggested that one possible way to avoid discoloration is to seal the dentinal walls of the access cavity with a dentin bonding agent. The recent review in dental traumatology does seem timely because there does appear to be a lack of evidence and conflicting results as well as a paucity of prospective studies.

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Corresponding Author

Dr. Behnam Bolhari, DDS, MSc.
Department of Endodontics,
School of Dentistry,
Tehran University of Medical Sciences,
Tehran, IRAN
Email id: b.bolhari@tumc.ac.ir