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Characteristics of materials used as cervical barrier in pulp revascularization: an integrative review

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• Conflicts of interest: none declared.

Abstract

Objective: to perform an integrative review on the characteristics of materials used as cervical barrier in pulp revascularization, addressing the bioactivity and biocompatibility, as well as the drawbacks inherent to their use. **Material and Methods:** searches were made, in English and Portuguese, on the platforms PubMed and Google Scholar. The following keywords were used: (pulp revascularization OR pulp regeneration) and (Biodentine OR Endosequence OR MTA Angelus OR Calcium Enriched Mixture OR ProRoot MTA) and (drawing OR discoloration) and (Biodentine OR Endosequence OR MTA Angelus OR Calcium Enriched Mixture OR ProRoot MTA). Inclusion criteria were: articles published from 2008 to 2018, *in vitro* studies, clinical studies, literature review and case reports. Exclusion criteria were: articles that did not address the topic of revascularization. **Results:** the electronic search found 337 articles. After title analysis, 81 articles were selected for reading the abstracts, and 66 articles were excluded according to the inclusion criteria. After complete reading of the 15 articles, 2 were excluded for not addressing the topic of this study. The journals found were: Dental Materials, International Endodontic Journal, Journal of Applied Oral Science, Journal of Dentistry of Tehran University of Medical Sciences, Journal of Endodontics and Journal of Medical Sciences, from 2010 to 2017. **Conclusion:** bioceramic materials used as cervical barrier showed bioactivity and reduced toxicity. As an inconvenience to its use, coronal discoloration is reported *in vitro* due to MTA, Biodentine and Endosequence, and *in vivo* only due to MTA. In addition, mineralized tissue may form inside the root canal, possibly due to the bioactivity of these materials.

Keywords: Bioceramic; Discoloration; Pulp revascularization.

Introduction

The treatment of necrotized teeth with incomplete rhizogenesis is still a major challenge for endodontists.[1] This interruption results in a deficiency in the crown-root proportion and in the thickness of dentinal walls[2]. Although the same principles that guide endodontic therapy of completely developed teeth are applied to these cases, the objective is more complex, since the apical closing by hard calcified tissue is sought, or even the complete root development.[1]

Apexification is the most commonly used treatment in necrotized teeth with incomplete rhizogenesis. Two techniques of apexification are described in literature: the use of intracanal calcium hydroxide-based medication to stimulate the formation of a hard tissue barrier, and the confection of a barrier with bioceramic material.[1,2] The first treatment has the disadvantage of increasing radicular wall weakening due to successive exchanges of calcium hydroxide paste, and a long time is necessary to form the barrier.[1] As for the confection of a barrier with bioceramic material, it does not require successive exchanges of measurement and allows less appointments. However, none of the techniques allows the continuity of root development.[1-3]

Considering the possibility of further root development and reinforcement of dentinal walls by hard-tissue deposition, strengthening the root against fracture pulp revascularization of necrotized teeth with incomplete rhizogenesis has been widely studied and proposed as a treatment modality. Pulp revascularization is characterized by the invagination of undifferentiated cells of the apical region in teeth of young patients with open apex.[3] The short treatment time and the reduced number of sessions are advantages of this therapy, when compared with the technique with intracanal medication exchange.[3]

The conventional protocol for the revascularization therapy is performed in two clinical sessions. Initially, the root canal system is decontaminated, usually chemically passively, using sodium hypochlorite (NaOCI) in a concentration between 2.5 and 6%,[2,3] followed by disinfection with the use of an triple antibiotic paste (ciprofloxacin, metronidazole and minocycline) as intracanal medication, and by the coronal sealing.[3] After these steps, in a following appointment, scheduled from 2 to 4 weeks after the first one, the removal of the medication and bleeding induction are performed, so that a clot is formed. This will allow, along with the growth factor, cell differentiation to form a new tissue. After the clot is formed, a bioceramic material is used as cervical barrier, a stage known as cervical sealing. Finally, definitive restorative materials are used for sealing the access cavity. [3,4]

To make the cervical sealing, a material is used as a physical barrier to contain the bleeding only in the root and to avoid new root canal infections. Thus, this material, besides biocompatibility, must present a good sealing capacity, even in the presence of moisture.[3] Among the materials used as cervical barrier, the MTA Angelus®, ProRoot MTA®, Biodentine®, Endosequence® and Calcium Enriched Mixture (CEM) stand out.[5]

Considering the different bioceramic materials used in

the cervical sealing with different compositions, this study aims to conduct an integrative review on the characteristics of the materials used as a cervical barrier in pulp revascularization, addressing the bioactivity and biocompatibility of the materials and the drawbacks found in literature regarding their use.

Material and Methods

To perform this study of integrative review, searches were made in English and Portuguese in the platforms PubMed and Google Academic. The following keywords were used: (pulp revascularization OR pulp regeneration) and (Biodentine OR Endosequence OR MTA Angelus OR Calcium-Enriched Mixture OR ProRoot MTA) and (darkening OR discoloration) and (Biodentine OR Endosequence OR MTA Angelus OR Calcium-Enriched Mixture OR ProRoot MTA). The inclusion criteria used were: articles published from 2008 to 2018, *in vitro* studies, clinical studies, clinical case reports literature reviews and systematic review, which addressed materials used for cervical barrier in cases of pulp revascularization/regeneration.

Articles published before 2008, in addition to those that did not address the procedure of pulp revascularization along with the use of materials for cervical barrier, were excluded of this study.

The final synthesis was developed in a descriptive form, considering the type of study, material and characteristic evaluated, results obtained from each of the studies. Such data were grouped by similarity and organized in thematic categories.

Results

The electronic search found 337 articles, and repeated articles were discarded from the total count. After analyzing these articles by title, 81 articles were selected for abstract reading. Of these, 66 were excluded because they did not address the specific thematic of this study. After completely reading 15 articles, 2 were excluded based on the inclusion criteria adopted, totaling 13 articles for this review.

Four *in vitro* studies addressing the bioactivity and cytotoxicity of the materials used as cervical barriers were selected.[6-9] Regarding the publication years and journals, one article from 2014 was found in the Journal of Endodontics,[6] one article from 2015 in the Dental Materials,[9] and two articles from 2017 in the International Endodontic Journal[7] and the Journal of Medical Sciences.[8] The findings are shown in Table 1.

In vitro studies and clinical cases addressed the draw-

Table 1. Evaluation of bioactivity and cytotoxicity of materials used for cervical sealing

Study	Type of study	Material evaluated in cervical sealing	Characteristic assessed	Results observed
Chang *et al.*, 2014[6]	*In vitro*	ProRoot MTA, Bioaggregate, MicroMega MTA	Biocompatibility and Odontogenic potential	ProRoot MTA, Bioaggregate e MicroMega MTA obtained equally good biocompatibility; regarding odontogenic potential, all materials promoted the formation of mineralization nodules and improved osteogenic/odontogenic markers.
Rodrigues *et al.*, 2017[7]	*In vitro*	MTA and MTA Plus	Cytotoxicity, Osteogenic bioactivity, mRNA expression of osteogenic markers, alkaline phosphatase and osteocalcin	Both did not show cytotoxicity and improved the expression of osteogenic markers. In addition, the MTA obtained greater areas of mineralization than the MTA Plus, and the alkaline phosphatase was greater in the MTA Plus group.
Mohamed *et al.*, 2017[8]	*In vitro*	MTA and CEM	Proliferation and viability of dental pulp stem cells	There was an initial decrease in cell viability in one day. Then, there was an increase in the cell count for MTA and CEM.
Bortoluzzi *et al.*, 2015[9]	*In vitro*	Biodentine, TheraCal and MTA Angelus	Viability and osteogenic differentiation of dental pulp stem cells	TheraCal obtained greater cytotoxicity. The osteogenic differentiation increased after exposure to Biodentine

MTA – Mineral Trioxide Aggregate; CEM – Calcium Enriched Mixture

backs found in revascularization therapy, as shown in Table 2.[10-18] Regarding the analysis of discoloration, a total of 8 articles, 4 case reports and 5 *in vitro* studies were found. Regarding publication years and journals, three case reports, from 2010, 2011 and 2017, were found in the Journal of Endodontics[10,11,17] and one article, from 2016, in the Australian Dental Journal.[16] Regarding *in vitro* articles, three articles in the Journal of Endodontics, from 2013[12] and 2015[14,15] were found, and one article from 2015 in the Journal of Applied Oral Science.[14]

Concerning the presence of intracanal calcification, one article from 2015 was found in the Journal of Dentistry of Tehran University of Medical Sciences.

Discussion

The pulp revascularization process in teeth with open apex, reimplanted or transplanted, has been reported in literature since the 1950s. Years later, this principle was taken to endodontics to treat necrotized teeth with incomplete rhizogenesis.[4] This treatment aims to eliminate symptoms, repair periapical lesions, and allow the continuation of root development.[19] According to the American Association of Endodontists (AAE), in 2013, the guidelines of regenerative treatment were reviewed, and the main objective of the treatment became healing the apical periodontitis, having as secondary objective the increase in root thickness/length and, finally, the recovery of the positive response of pulp tests, since the secondary objectives are not always achieved.[18]

This study previously described a treatment protocol, named in the conventional protocol text. However, other treatment approaches are described in literature, such as decontamination of the canal system with chlorhexidine 2% as auxiliary chemical substance; the use of chlorhexidine associated with calcium hydroxide and double antibiotic paste as medication between sessions, and, still, in some protocols, this therapy has been preconized in a single session.[2,3,10,18]

Among pulp revascularization stages, this study assessed the use of different materials used as cervical barrier. Of the materials evaluated in this study, articles were found using white and gray MTA,[6-16] Biodentine,[9,14,15,18] EndoSequence,[14,15] TheraCal,[9] and the Calcium-Enriched Mixture (CEM).[4,8,17]

The materials used in cervical sealing act as physical barrier, protecting the clot and preventing possible recontamination. Also, in this stage, bioactive materials facilitate the differentiation of mesenchymal stem cells to produce new dental tissues. Regarding the osteogenic potential/bioactivity, the only material that showed greater mineralization power was the MTA, when compared with MTA Plus. The other materials showed a similar osteogenic potential.

Regarding biocompatibility, the ProRoot MTA, Bioaggregate, MicroMega MTA, Biodentine, CEM, MTA and MTA Plus obtained reduced cytotoxicity results. The most toxic material was the TheraCal, and Bortoluzzi *et al.*[9] explained that it is due to the monomers of the formulation components of this bioceramic cement, which accumulate over time and end up generating cell apoptosis. However, this material is indicated for indirect, and direct pulp capping and a base and is not routinely used as a cervical barrier.

MTA was the material initially preconized and used as cervical barrier, being considered the first generation of bioceramics. One believes that the MTA success is due to its high sealing capacity, biocompatibility and potential for promoting the formation of a mineralized tissue.[6] However, due to its poor physical properties, which include difficult manipulation and insertion in the proper place and prolonged setting time, associated with the need for moisture to set, new bioceramic materials started to be investigated, assessed and indicated as MTA improvement.[9,21] In addition, Dabbagh *et al.*[22] report they are inconvenient for the use of MTA, because this material moves into the clot during condensation. To work around the drawbacks related to material placement and its association with the clot, the use of a collagen barrier immediately after the clot is formed has been suggested to limit the clot position and serve as bulkhead to place the MTA.[11,23]

In addition to the disadvantages listed below, another aspect found in literature is coronal discoloration. This was initially attributed to medication, due to the presence of minocycline.[11] However, in previous studies with calcium hydroxide as intracanal medication and MTA as cervical barrier, tooth discoloration was observed.[24,25] Thus, this article is also focused on assessing the coronal discoloration caused by different materials used for cervical sealing. As observed in Table 2, the MTA, regardless of the formulation, that is, gray and white, may lead to tooth discoloration. This inconvenience, according to literature, may be caused by oxidation and incorporation of iron oxide in the remaining MTA powder, or by the interaction between erythrocytes and the MTA.[10]

One advantage in the use of new bioceramic materials, which emerged as an improvement to MTA, is associated with reduced setting time and manipulation characteristics,

Table 2. Evaluation of the presence/absence of complications related to bioceramic materials in cases of pulp revascularization

Study	Type of study	Material evaluated in cervical sealing	Presence/Absence of complications	Follow-up time
Timmermar *et al.*, 2017[10]	Case report	MTA	Occurrence of discoloration.	1 month
Petrino *et al.*, 2010[11]	Case report	White MTA	Occurrence of discoloration.	10 days
Felman *et al.*, 2013[12]	*In vitro*	White MTA	Occurrence of discoloration.	of 1 and 35 days
Santos *et al.*, 2017[13]	*In vitro*	TAP+ White MTA, TAP + Glass-ionomer cements	TAP/MTA showed significantly greater discoloration than TAP/CIV	2 weeks
Beatty *et al.*, 2015[14]	*In vitro*	Biodentine, Endosequence, ProRoot MTA	Biodentine, Endosequence and ProRoot MTA discolored, however, the Endosequence and Biodentine change increased after 8 weeks, and the ProRoot change stabilized 1 day after its placement.	8 weeks
Shokouhinejad *et al.*, 2015[15]	*In vitro*	Endosequence Ortho MTA, Biodentine ProRoot MTA	There was color change, and the greater change occurred in the presence of blood associated with Ortho MTA, and a smaller one in Biodentine, followed by Endosequence in the absence of blood.	6 months
D'Mello *et al.*, 2016[16]	Case report	White ProRoot MTA	Occurrence of discoloration.	7-36 months
Nosrat *et al.*, 2011[17]	Case reports	CEM	In the first case, complete root formation was observed in the distal root and thickening only in the walls of the mesial root. Complete root formation in the second case. Periapical health in both cases. Discoloration was not observed with the use of CEM.	15-18 months
Khoshkounejad *et al.*, 2015[18]	Case report	Biodentine	Success according to the AAE for apical health. However, no evidence of canal wall thickening, and continuity of root development was observed. Diffuse intracanal calcifications and radiopaque calcified bridges in the middle and apical canal parts were observed	6 months - 1 year

TAP- Triple antibiotic paste; MTA- mineral trioxide aggregate, CEM- calcium calcium-enriched; AAE – American Association of Endodontists.

when compared with MTA, which presents an extensive setting time associated with the need for moisture.[22,26] Regarding these bioceramic materials, the discoloration generated by them was evaluated *in vitro* through spectrophotometry. As a result, as shown in Table 2, Biodentine, Endosequence and OrthoMTA showed tooth discoloration, when assessed in an *in vitro* study using a device simulating the revascularization technique, which consisted of the use of these materials on a foam saturated with blood, similar to ProRoot MTA. In the absence of blood, Biodentine and Endosequence discoloration was significantly smaller than OrthoMTA. After 6 months, a color change significantly larger occurred in the OrthoMTA contaminated with blood, while a significantly smaller color change was observed in Biodentine, followed by Endosequence in the absence of blood ($p<0.05$). No clinical studies or case reports of the new bioceramic showing tooth discoloration with the use materials were found. As for MTA, both in *in vitro* studies and case reports, discoloration was observed.

To prevent it, Shokouhinejad *et al.*[15] proposed the use of an adhesive system before placing the triple antibiotic paste during pulp revascularization. However, the authors observed that this sealing reduced the degree of discoloration, but it did not eliminated it. When discoloration is observed, internal whitening is an effective treatment option.[10]

Mineralized tissue/calcification was observed in the middle and apical canal parts in the study by Khoshkounejad *et al.*[18] and may be related to the potential of tissue induction by bioceramic materials.[27,28] Shabahang *et al.*[29] state that this phenomenon is similar to what happens when a MTA

plug is apically used in apexification treatments. This fact may occur because it is a defense mechanism to separate the contaminated root canal from the more internal periapical tissues.[28]

Due to its use as a physical barrier, preventing contamination, we used as search terms the association between the words "sealing" OR microleakage associated with the different materials used to this end, and the terms revascularization and regeneration. However, no findings were made in the search evaluating sealing/leakage of the different materials used as cervical barriers in pulp revascularization. Thus, the association between these terms was removed from the methodological criteria used in this study. We emphasize, however, the need for further *in vitro* articles evaluating the sealing in different materials used as cervical barrier.

This integrative review assessed the different materials used as cervical barrier and verified that they have bioactivity and reduced toxicity.[6-9] As a drawback to its use, coronal discoloration was verified, associated with the use of *in vitro* MTA and case reports,[10-16] and Biodentine[14,15,18] and Endosequence *in vitro*.[14-15] Articles evaluating the discoloration of other bioceramic materials *in vitro* and *in vivo* were not found. Studies are necessary to assess the sealing efficacy of materials used as cervical barrier.

In Brazil, MTA has been marketed for a few years, with a reduced cost when compared with Biodentine, recently launched in the national market. The dentist need to know the characteristics of the different materials available in the market, based on scientific evidence, to choose the best material for their clinical practice.

Conclusion

Based on the articles selected, the conclusion was that bioceramic materials used as cervical barrier have bioactivity and reduced toxicity. As an inconvenience to its use, coronal discoloration is reported *in vitro* due to MTA, Biodentine and Endosequence, and *in vivo* only due to MTA. In addition, mineralized tissue may form inside the root canal, possibly due to the bioactivity of these materials.

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