Material Pulp Cells and Tissue Interactions

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SIGNIFICANCE

This review summarized the osteoinductive, biocompatible, anti-inflammatory, antimicrobial, and esthetic properties of various biomaterials suitable for vital pulp therapy and regenerative endodontic procedures.

ABSTRACT

Two increasingly common endodontic procedures, vital pulp therapy (VPT) and regenerative endodontic procedures, rely on dental tissue regeneration/repair mechanisms with the aid of biomaterials. These materials are applied in close contact to the pulpal tissue and are required to be biocompatible, form an antimicrobial seal, not induce staining, and be easy to manipulate. Historically, calcium hydroxide played an important role in VPT. However, over the last 3 decades, significant efforts in research and industry have been made to develop various biomaterials, including hydraulic tricalcium silicate cements. The present review summarized various hydraulic tricalcium silicate cements and their biological properties in clinical procedures, namely VPT and regenerative endodontic procedures. *(J Endod 2020;46:S150–S160.)*

KEY WORDS

Biomaterials; pulp capping; pulpotomy; regenerative endodontics; tissue interaction; vital pulp therapy

Bioactive materials are inevitable components of modern-day dentistry. This is particularly evident from the growing body of evidence that supports dental tissue regeneration as a viable modality of treatment. Therefore, given the wide range of regenerative applications, the aim of many companies and research laboratories has been to generate a material that is biocompatible and osteoinductive, does not induce tooth staining, and is easy to manipulate. Two such clinical procedures that use bioactive materials are vital pulp therapy (VPT) and regenerative endodontic procedures (REPs). This review summarizes the salient features of various bioactive materials in the previously mentioned clinical applications.

VPT

Caries, trauma, and iatrogenic errors can result in contamination of the pulp with microbes stimulating pulpal inflammation. The pulp has an excellent capacity to heal if the irritant is removed in a timely manner. Therefore, the International Caries Consensus Collaboration recommends treating deep cavities biologically by means of selective caries removal to the deepest soft dentin in order to avoid pulp exposure[1]. Hence, VPT, a minimally invasive endodontic treatment strategy, was developed. However, if left untreated, irreversible damage ensues within the pulp tissue[2]. With regard to immature permanent teeth, the primary goal is to preserve the pulp in order to promote further root development[3] and reduce the risk of cervical and vertical root fractures[4]. In mature permanent teeth, irreversible pulpitis is commonly treated by eradication of the entire pulp and root canal treatment (RCT). Nevertheless, RCT is costly and time-consuming and requires considerable compliance from patients, especially when they are young[5,6]. Moreover, a recent histologic and microbiological study has undermined our diagnostic decision making and traditional treatment management of nonsurgical root canal treatment for teeth with irreversible pulpitis. This study reported no bacterial invasion of the radicular pulp in teeth diagnosed with irreversible pulpitis[7]. However, these tissues undergo changes leading to the loss of odontoblasts, irregular and diffuse calcifications, and collagen deposition. The majority of the radicular tissues were not inflamed, suggesting a pulp-induced repair process[8]. Furthermore, this adds to the body of evidence that currently available diagnostic tools lack accuracy and that clinical symptoms cannot be accurately correlated to the histologic status of the pulp[9]. This diagnostic gray area along with our improved understanding of pulpal disease has led to an increase in the use of VPT treatments, namely pulp capping and partial or full pulpotomies.

A microbial seal is paramount to the success of VPT. Additionally, protection of the radicular pulp from unreacted monomers from resin-based restorative materials also calls for the use of biocompatible materials that would serve as a protective layer between the toxic components of restorative materials and the pulp-dentin complex[10]. Therefore, a biocompatible material must possess properties of adequate sealing as well as serve as an antimicrobial agent[11,12]. Historically, calcium hydroxide ($Ca[OH]_2$) played an

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0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2020.06.031

important role in the evolution of VPT. It is antimicrobial and has the ability to extract growth factors from mineralized dentin, inducing reactionary dentin deposition[13]. However, clinical outcomes of VPT were considered unpredictable primarily because of the nonspecific mechanism of action, the lack of sealing properties, and varied hard tissue formation with calcium hydroxide $Ca(OH)_2$[14]. More recently, with the advent of a new class of materials, namely hydraulic tricalcium silicate cements (hTCSs) and a mixture of calcium silicates and calcium phosphates, clinical success has greatly increased (Table 1). This is because of the excellent biocompatibility and bioactivity potential of this class of materials[20,21]. The prototypical hTSC was introduced in the 1990s—mineral trioxide aggregate (MTA)[22]. However, in the following decades, Portland cement, calcium-enriched mixture (CEM; BioniqueDent Yektazist Dandan, Iran Polymer and Petrochemical Institute, Tehran-Karaj, Iran; US Patent no. 20080206716), Biodentine (Septodont Inc, Saint-Maur-des-Fossés, France), EndoSequence BC (Brasseler USA, Savannah, GA), and BioAggregate (Verio Dental Co Ltd, Vancouver, Canada) have been developed and applied as well[23]. Several of these materials have been developed to overcome the long setting times of MTA. The setting times of these hTCS are mentioned in Table 1.

REPS

Over the past 2 decades, a novel endodontic treatment modality offers several unique advantages for teeth with an immature apex and pulp necrosis. REPs aim at resolution of disease, restoring the lost immunocompetent tissues, and generation of undeveloped tissues such as the completion of root development and its associated pulp[24]. The American Association of Endodontists (AAE) and the European Society of Endodontology have respectively published clinical considerations and a position statement in order to achieve a standardization based on the available evidence[25,26]. As outlined by the AAE, the goal of this regenerative therapy is 3-fold:

(1) Resolution of signs and symptoms of apical periodontitis,
(2) Root development, and
(3) Re-establishment of vitality responses.

Conceivably, a greater regenerative goal is desired in REPs than in VPT. Therefore, it is imperative that materials in contact with incoming stem cells provide not only an adequate seal but also serve as a potential source of signaling molecules that regulate inflammation and promote mineralization.

TABLE 1 - The Setting Times of Commonly Used Biomaterials

Biomaterial	Setting time
Mineral trioxide aggregate (MTA)	2 h 20 min (white MTA[15])–2 h 45 min (gray MTA[15])
Portland cement	2 h 20 min–2 h 45 min similar to MTA[15]
Biodentine	12 min[16]
Calcium-enriched mixture	50 min[17]
Endosequence BC	20 min[18]
BioAggregate	4 h[19]

PROPERTIES OF BIOMATERIALS

Osteoinductive Properties of Commonly Used Materials

A bioactive material elicits a biological response from a living tissue resulting in a bond between the tissue and the material[20,27]. More specifically, when a bioactive material is immersed in a serumlike solution, it forms a carbonated apatite, being the biological apatite found in bone, cartilage, enamel, and dentin[28,29]. As mentioned previously, a significant number of hTCSs have been developed over the last 30 years[23]. They may vary in chemical composition; however, they are all known for their biocompatibility and bioactivity potential[20,21,23]. The apatite-forming ability of materials immersed in simulated body fluid has been investigated *in vitro* according to ISO standards (ISO 23317 2012)[30]. Also, aging of hTCSs in simulated body fluid and other modifications of physiological fluid (Hank's balanced salt solution, Dulbecco phosphate-buffered saline, etc) has already been tested *in vitro*. These studies showed the bioactivity and hence the osteo- or hard tissue formation with hTCSs[31,32]. However, regarding the few patient samples analyzed so far, the *in vivo* bioactivity of hTCSs in endodontic treatment modalities seems to appear less than ideal[18,33], possibly because of the lack of a controlled environment. Hence, atmospheric carbon dioxide, blood and bacterial contamination, and specimen preparation protocols may affect the bioactivity potential of the hTCSs and may produce calcium carbonate rather than carbonated apatite observed in patient samples[18,33]. Nevertheless, this presumed apatite-forming ability of hTCSs is commonly appreciated at the root end, more specifically when it is placed as an apical seal in close contact with the medullary bone during root-end surgery and apexification[34]. However, bone induction is undesirable inside the root canal. hTCSs are placed coronally during VPT and REP in close contact with stem cells or cells responsible for mineralization such as odontoblastlike cells, forming a coronal seal but also inducing mineralization. This mineralized bridge with cellular inclusions (Fig. 1*A* and *B*) below the hTCS often remains discrete in volume and is seen as an additional seal in the coronal third of the root canal[35]. This calcified barrier induced by the biomaterials has been described as cementumlike tissue or osteodentin[36–39]. The bridge induced by MTA was shown to be tighter than the porous calcified layer produced by $Ca(OH)_2$[40]. It has previously been described that the hard tissue bridge formed in apexification cases with $Ca(OH)_2$ is coronally and apically incomplete because of vascular inclusions[41], which can lead to bacterial invasion. On the other hand, MTA has an excellent cementum- and periodontal ligament–inducing potential[42–44] and creates a tight seal and a hard tissue bridge quicker and with less cellular inclusions than $Ca(OH)_2$[40,41,45]. Nevertheless, it is noteworthy that in a few failed REP cases (because of sequelae of trauma) in which hTCSs were applied, this mineralization becomes invasive, filling nearly the entire root canal with a layered pattern suggestive of a continuous turnover process[46] (Fig. 2*A* and *B*). Although this represents an undesirable histologic outcome, this is an acceptable clinical outcome. Further research is warranted to investigate if the "hypermineralization" (Fig. 2*A* and *B*) is a result of an ongoing inflammatory process. Other insights into this might be material-specific induction of ectopic osteoidlike tissue. A recent study by Miller et al[47] showed that stem cells from the apical papilla survived and differentiated into a dentinogenic phenotype when in contact with Biodentine and EndoSequence Bioceramic Root Repair Material (Brasseler USA). On the other hand, MTA shifted the differentiation of stems cells from the apical papilla into a more osteogenic phenotype[48]. This beneficial property of Biodentine over MTA can be attributed to its greater release of calcium ions[49] as well as critical growth factors such as transforming growth factor beta 1[50]. These superior effects over MTA have also been shown with BioAggregate[51]. Collectively, biomaterial selection is key to a successful histologic and clinical outcome.

Biocompatibility

A primary requirement for materials that interface with vital host tissue is to be nontoxic. hTCSs have undergone varying tests *in vitro* as

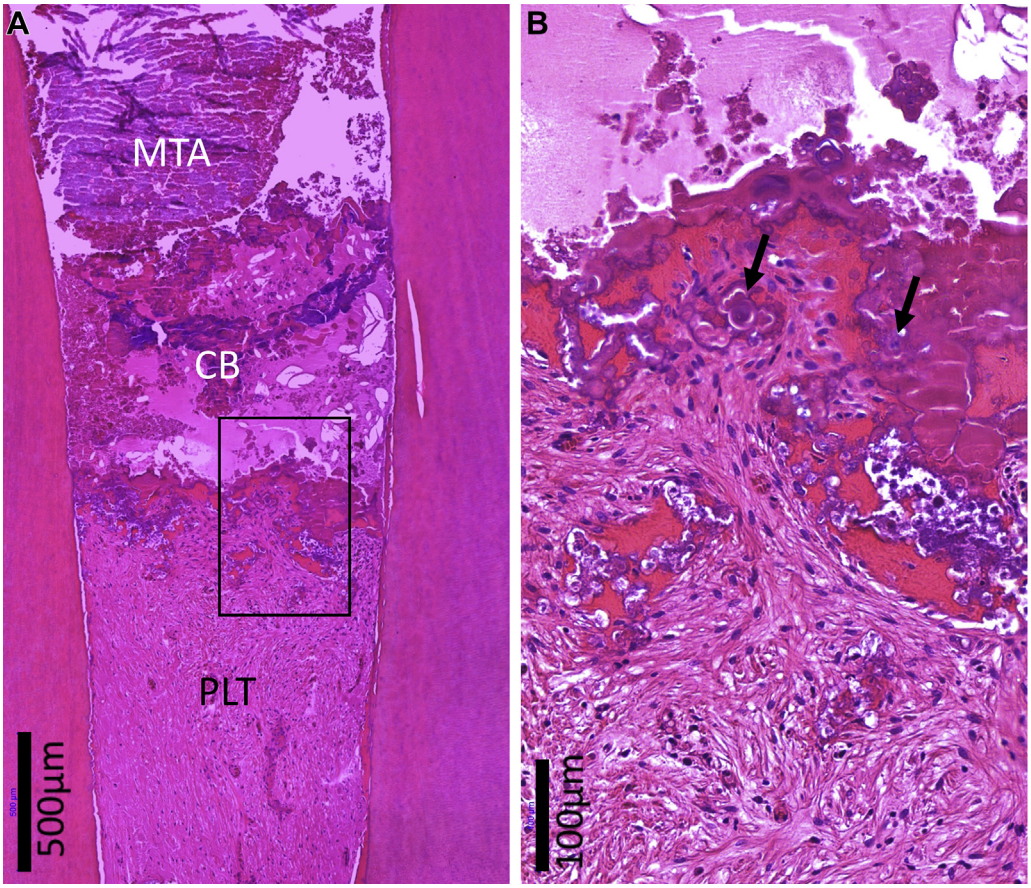

FIGURE 1 – Histologic analysis of an infected immature permanent right lower premolar extracted 11 months post-REPs. (*A*) Hematoxylin-eosin staining of a longitudinal slice of the tooth presenting from coronal to apical, MTA, a calcified bridge (CB), and pulplike tissue (PLT). (*B*) Magnification of the *black rectangle* in *A*; the *black arrows* point to cellular inclusions inside the CB. Scale bars: (*A*) 500 µm and (*B*) 100 µm[31].

well as *in vivo* including general toxicity profile in a cell culture, implantation tests, and usage tests in experimental animals following accepted protocols[52]. hTCSs were not cytotoxic to human periodontal ligament fibroblasts[19]. Similarly, interactions with human dental pulp stem cells showed good cell viability, attachment, and migration. Additionally, genotoxicity tests also demonstrate nonmutagenic properties[53,54]. Moreover, MTA has been long known to induce oseteoblastlike cell differentiation in human dental pulp stem cells[55]. Additionally, Biodentine has also been shown to enhance the proliferation, migration, and adhesion of human dental pulp stem cells[56] as well as differentiation to odontoblastlike cells, which is regulated via the mitogen-activated protein kinase and Ca^{2+}/calmodulin-dependent protein kinase II pathways[57]. Furthermore, hTCSs have been shown to up-regulate gene expression of alkaline phosphatase, vascular endothelial growth factor, and other genes[55] to promote tissue repair.

Inflammation

An initial inflammatory reaction is an important part of the reparative and regenerative process of the dental pulp[58]. To this end, the effects of bacterial by-products such as lipopolysaccharide (LPS) or sodium hydrate, another factor released by gram-negative anaerobic bacteria, show altered differentiation of periodontal ligament stem cells and dental follicle stem cells[59,60]. Additionally, dental pulp stem cells subjected to high concentrations of LPS from *Porphyromonas gingivalis* show reduced levels of alkaline phosphatase and bone sialoprotein gene expression[61]. Therefore, for an ideal regenerative outcome, a timely attenuation of the inflammatory response is critical.

Currently available biomaterials possess anti-inflammatory properties that make them attractive restorative materials. This is evident with hTCSs that promote lower levels of inflammatory mediators such as interleukin-1α, -1β, -2, and -6 when in contact with mineralizing cells *in vitro*, suggesting an anti-inflammatory role[62]. Moreover, hTCSs, namely Biodentine, increase fibroblast-induced complement C5a, which has recently been shown to provide a critical balance between pulp inflammation and regenerative potentials[63]. These findings were replicated in a clinical study comparing hTCSs versus resin-containing biomaterials, namely TheraCal LC (Bisco, Schaumburg, IL). Specifically, findings from this study show that both Biodentine and MTA performed significantly better with respect to postoperative pain as well as the minimal inflammatory response post-VPT[64]. Moreover, when compared with $Ca(OH)_2$, hTCSs form a more uniform calcific bridge with less inflammation in the surrounding tissues as well as a good seal[65]. Other anti-inflammatory actions of hTCSs such as Biodentine and Angelus MTA (Angelus Indústria de Produtos Odontológicos, Londrina, Paraná, Brazil) include lower levels of nitric oxide and prostaglandin E_2 production upon interaction with the dental pulp detected via an enzyme-linked immunosorbent assay compared with Immediate Restorative Material (Dentsply

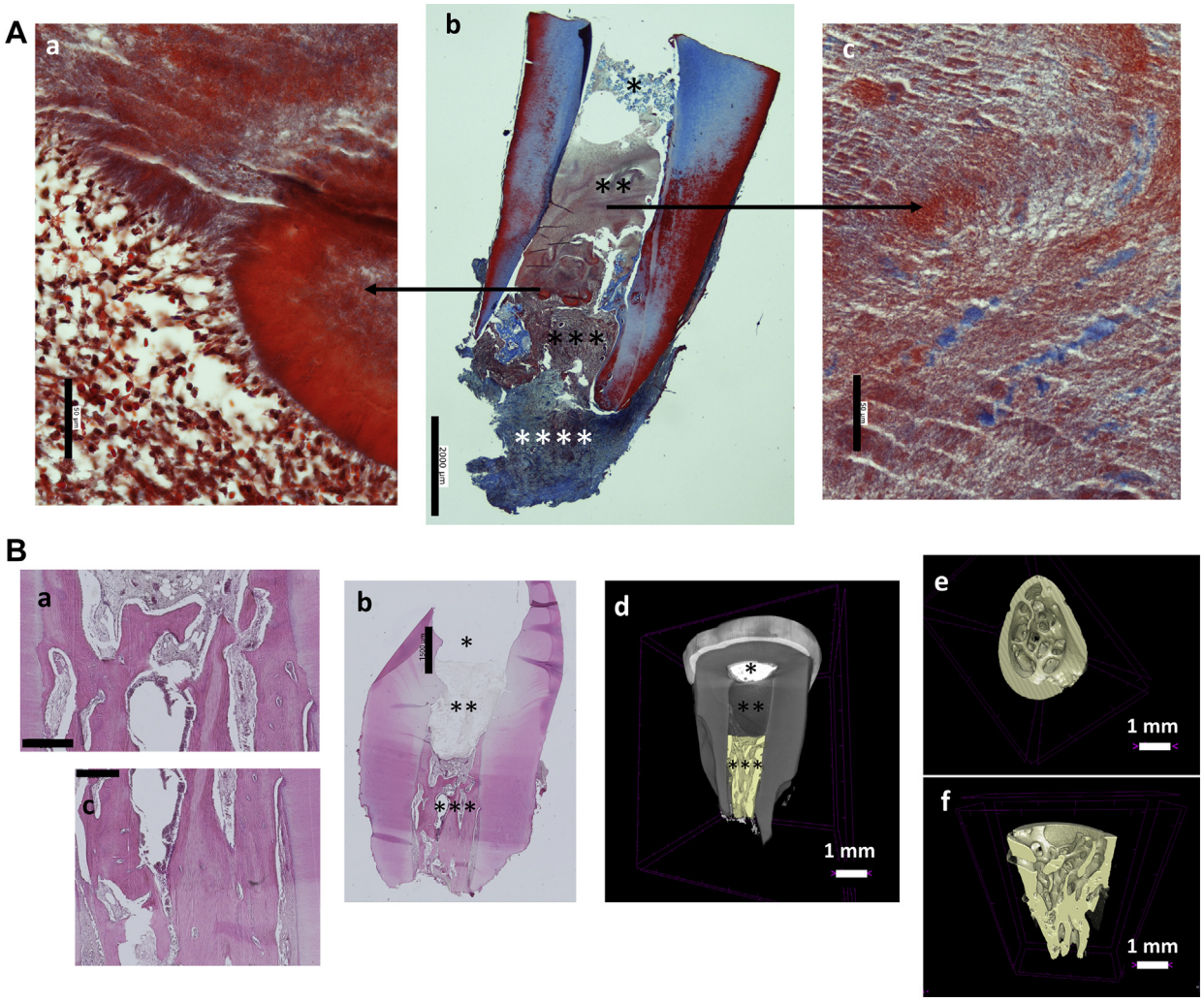

FIGURE 2 – Biomaterial-induced hypermineralization after application of Portland cement (Medcem, Vienna, Austria) as the hTCS in the REP. (*A*) Trichrome von Masson–stained histologic slices of an extracted central upper incisor. The extraction was because of a persistent periapical infection 7 months after REP. (*Aa*) A magnified section of *Ab* presenting layered calcification. (*Ab*) From coronal to apical, Portland cement (*), calcification (**), pulplike tissue (***), and an apical granuloma (****). (*Ac*) Layered calcification, suggesting a circadian clock rhythm. Scale bars: (*a* and *c*) 50 µm and (*b*) 2000 µm (unpublished data from ISO 23317-2[30]). (*B*) An avulsed right upper central incisor of a 9-year-old boy received REP because of inflammatory root resorption. Fifteen months after REP, the tooth was extracted because of insufficient root development and uncertain orthodontic prognosis despite periapical bone healing. (*Bd–Bf*) A nano–computed tomographic scan was taken, and (*Ba–Bc*) a histologic analysis (hematoxylin-eosin staining: *Ba–Bc*) was performed. (*Bb–Bd*) From coronal to apical, glass ionomer cement (*), Portland cement (**), and pulplike tissue with hypercalcification (***). (*Ba*) The coronal third of the root canal, (*Bc*) the midradicular region, and the (*Be*) coronal and (*Bf*) transversal views of the mineralization inside the root canal. Scale bars: (*Ba* and *Bc*) 400 µm, (*Bb*) 1500 µm, and (*Bd–Bf*) 1 mm. (From Meschi N, Schol N, Van Gorp G, et al. Cellular network in situ and spatial organization of a revitalized tooth: preliminary results of a histological and microradiographical analysis. Poster presented at: IADR PBRG Symposium. June 23–25, 2019; Portland, OR.)

Sirona, York, PA)[66]. Messenger RNA expression profiles of several inflammatory cytokines such as tumor necrosis factor alpha and interleukin -1β , -6, and -8 was lower when compared with IRM[66]. Taken together, these findings suggest a strong immunomodulatory role of bioceramics when in contact with cells critical for regeneration.

Antimicrobial Properties

Microbial contamination has long been known to be detrimental to the pulp regenerative potential. Studies evaluating this phenomenon have shown that biofilms cause reduced migratory and differentiation capacities of bone marrow stem cells[67]. Moreover, in procedures such as REPs, little to no mechanical debridement of the canal system is recommended; therefore, it is likely that bacteria are incompletely removed. Indeed, REP studies performed on dogs[68] and ferrets[69] show that 30%–50% of teeth had persistent viable bacteria even after 2 weeks of treatment with triple antibiotic paste. Additionally, the histologic outcome of human teeth extracted post-REP has shown less than ideal success with hard tissue regeneration[35,70–73]. These studies shed insight into the importance of adequate disinfection for an ideal regenerative outcome.

The majority of hTCSs have a high pH in the range of 10.2–12.5 during the initial setting of the material. This high pH provides antimicrobial action against common endodontic pathogens[74]; this together with their bioactivity properties[75,76] makes hTCSs desirable materials for endodontic microsurgeries[77]. ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK) and bioceramics have been shown to have an antibacterial effect against planktonic *Enterococcus faecalis*[78], and a more recent study has shown that both

materials have comparative antifungal biofilm activity[79]. The antimicrobial effect of EndoSequence BC Sealer, a sealer version of EndoSequence BC putty (Brasseler USA), has been shown against an 8-week-old *E. faecalis* biofilm[78]. This further strengthens the use of bioceramics as antimicrobials, thereby promoting the goal of regeneration.

Esthetic Outcomes

Tooth discoloration was 1 of the major drawbacks of earlier hTCSs[80]. This was attributed to the radio-opacifier bismuth oxide in MTA[81] (Fig. 3*A*). Newer hTCSs have attempted to reduce the risk of tooth discoloration while attempting to improve biocompatibility[82] (Fig. 3*B*). Later formulations of MTA replaced bismuth oxide with tantalum oxide[83], whereas Biodentine (Fig. 3*C*) has zirconium oxide and EndoSequence BC putty and sealer have zirconium oxide and tantalum oxide as a radio-opacifier[84,85]. Moreover, a recent study comparing coronal tooth discoloration with various hTCS materials using spectrophotometry found discoloration with gray and white MTA; however, no discoloration was observed with Biodentine or EndoSequence BC putty[86]. Restorative management has been suggested to reduce discoloration associated with MTA; these include the removal of material and bleaching[87].

CLINICAL OUTCOMES OF VPT

Systematic reviews present no significant differences between $Ca(OH)_2$ and MTA as VPT agents. Both materials provide satisfactory outcomes[88,89]. Aguilar and Linsuwanont[88] calculated a weighted pooled success rate of each VPT type ranging between 72.9%–99.4%, providing evidence that vital permanent teeth with cariously exposed pulps might be managed successfully by VPT. However, Bjorndal et al[90] recognized 2 general types of failure: early and long-term. The early failures came up shortly after the VPT and might have been related to underestimation of the severity of the pulpitis (ie, misdiagnosis). On the other hand, the failures reported several months post-VPT might have been caused by loss of the coronal seal or absence of calcified bridge induction. The outcomes discussed until now are based on VPT performed on deeply decayed immature permanent teeth with no symptoms or reversible pulpitis. Nevertheless, in a growing number of clinical trials, VPT instead of pulpectomy was performed to cure biologically immature permanent teeth with irreversible pulpitis[91,92]. In a few systematic reviews, the outcome of these trials has been assessed; 8

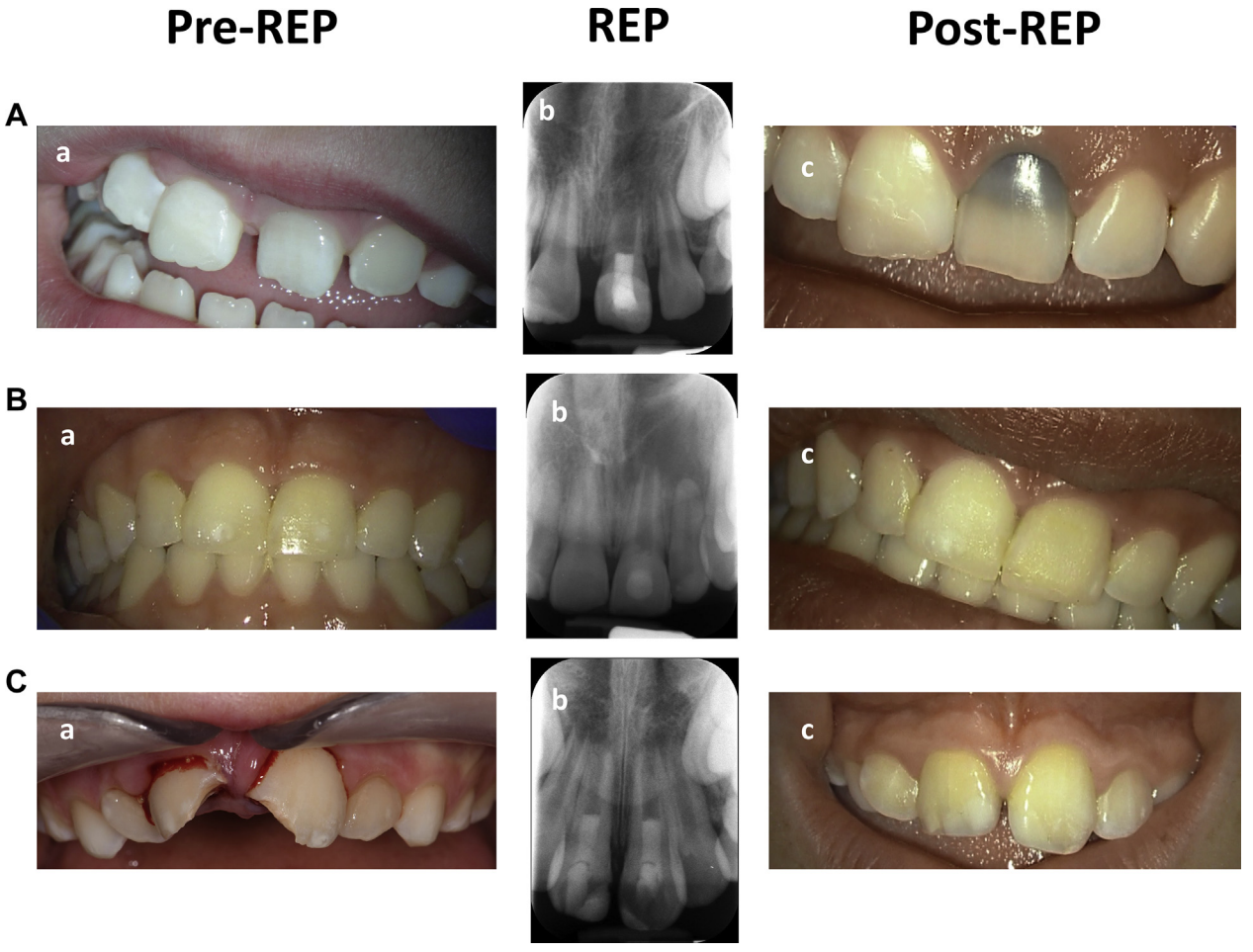

FIGURE 3 – The discoloration induced by hTCSs. (*Aa*) A 6-year-old girl presenting a lateral luxation of the upper left central incisor. (*Ab*) REPs have been performed with white MTA (ProRoot). (*Ac*) A clinical image 7 years after REP presents black staining of the REP tooth. (*Ba* and *Bb*) An 18-year-old man presenting a periapical infection on the upper left central incisor caused by an enamel-dentin fracture many years ago. REP was performed with Portland cement (Medcem) as the hTCS. (*Bc*) Three years later, the treated tooth presented a slight yellowish discoloration. (*Ca*) An 8-year-old boy presenting an enamel-dentin fracture with pulp involvement on both upper central incisors. (*Cb*) REP was performed on both teeth with Biodentine as the hTCS. (*Cc*) Six months after REP no discoloration was visible, but a long-term follow-up is necessary in order to assess this adequately.

heterogenous studies with a high risk of bias presented a clinical success rate of 97.4% and a radiographic success rate of 95.4% after 1 year[91,93]. Remarkably, these rates dropped to 94% and 88.4%, respectively, at the 3-year follow-up[91]. A randomized controlled clinical trial using CEM in 4 types of VPT in mature permanent teeth with irreversible pulpitis presented favorable/comparable outcomes after 1 year[92]. Because of these results, VPT is being seen as a prospective substitute for pulpectomy; however, more trials comparing VPT with alternative treatments are necessary to assess the cost-effectiveness[93].

CLINICAL OUTCOMES OF REPS

Most of the clinically applied REP are based on homing of mesenchymal stem cells into the root canal via triggering a blood clot[94]. Similar to VPT, hTCSs are applied in REPs as well as coronal plugs[25,26]. Hence, in the coronal third of the root canal, a mineralized bridge is mostly induced by the biomaterial placed on the blood clot[35,46,95–97]. However, the intracanal mineralization observed post-REP might be from a different source, such as ingress of bone marrow stem cells or periodontal ligament stem cells. In many cases, apical ingrowth of periodontal tissue is visible[35,46,70–72,95–99]. In nearly 85% of the reported REP cases, MTA has been used as a coronal plug next to CEM cement, Biodentine, and EndoSequence BC putty[100]. Hence, REP with MTA has the greatest evidence base and has been reported to be successful, as seen with the resolution of apical periodontitis (first success criteria of the AAE and European Society of Endodontology)[94]. High pooled survival (97.8%) (average follow-up = 16.7 months) and success (91.3%) rates have been reported, showing no significant difference between REP and MTA apexification[94]. Nevertheless, further root development (second success criteria) of cell homing–based REP teeth might take years and be incomplete in comparison with the contralateral sound teeth[95,96]. High-quality randomized controlled trials presenting longer follow-up periods are necessary to assess the survival of REPs with hTCSs in comparison with MTA apexification because the latter presents viable and predictable long-term tooth survival (up to 15 years postoperatively) without fractures[101].

CONCLUSIONS AND FUTURE DIRECTIONS

The last 2 decades have advanced our understanding of pulpal biology and the ability to use advancements in endodontic materials to improve outcomes. We require more robust randomized clinical trials to assess accurately the outcome of different types of VPT on different types of teeth (deciduous/permanent and mature/immature) and the type of biomaterial applied. Additionally, more randomized trials are required to investigate if VPT is a less time-consuming and cost-effective alternative to RCT for mature permanent teeth with irreversible pulpitis. More trials are required to assess the long-term survival of REP teeth because it is not known if they survive until the patient's full skeletal growth and hence are a viable alternative to MTA apexification.

Future development of materials looks promising with research ongoing targeting improvement in various areas. Some improvements would be faster setting times, improved handling, and no discoloration.

Nanoparticles could be used to enhance the physical properties and penetration of hTCSs[53]. The use of electrospun poly(-caprolactone) fiber meshes with hTCSs has been proposed with the aim to guide the regeneration of pulp tissue and calcific bridge[102]. The incorporation of various compounds have been suggested. Epigenetic therapeutics such as histone deacetylases inhibitors could alter the mineralization response to human dental pulp stem cells[103]. Simvastatin could be used to enhance calcific bridge formation[104] and N-acetylcysteine to enhance the antibacterial effect and aid in the differentiation of human dental pulp stem cells[105–107]. Pharmacologic inhibitors such as glycogen synthase kinase inhibitors have been proposed because of the ability to promote reparative dentin formation[108]. hTCSs have enhanced VPTs and will no doubt play an important role in the future of endodontics. However more accurate randomized controlled trials are needed to evaluate if these materials are suitable candidates to specifically enhance VPTs.

ACKNOWLEDGMENTS

Nastaran Meschi and Biraj Patel contributed equally to this study.

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

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