Are Premixed Calcium Silicate–based Endodontic Sealers Comparable to Conventional Materials? A Systematic Review of *In Vitro* Studies

Luiza Helena Silva Almeida, MS, DDS, Rafael Ratto Moraes, PhD,* Renata Dornelles Morgental, PhD,*† and Fernanda Geraldo Pappen, PhD**

Abstract

Introduction: This study aimed to compare the physicochemical and biological properties of premixed calcium silicate–based endodontic sealers with other conventional root canal filling materials by systematically reviewing laboratory studies. **Methods:** The search was conducted in 3 databases (Medline via PubMed, Scopus, and Web of Science) following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Two reviewers independently selected the studies and extracted the data. The properties of interest were bond strength, radiopacity, pH, solubility, setting and working time, dimensional change, flow, calcium ion release, antimicrobial activity, biocompatibility, and cytotoxicity. **Results:** From 2636 potentially eligible studies, 31 were selected for full-text analysis, and 27 were included in the review. Premixed calcium silicate–based endodontic sealers followed the ISO 6876:2012 requirements for most physicochemical properties except for solubility. The target sealers also presented favorable biological findings when compared with conventional sealers. **Conclusions:** Despite the lack of well-designed long-term clinical trials, the target premixed calcium silicate–based sealers show good physicochemical and biological properties *in vitro*. In general, the results were similar or better than conventional endodontic sealers as observed in *in vitro* and *in vivo* animal studies. *(J Endod 2017;43:527–535)*

Key Words

Calcium silicate-based sealer, EndoSequence BC, iRoot SP, root canal filling material, root canal sealer

Significance

There are still few *in vitro* studies and no long-term clinical trials about premixed calcium silicate–based endodontic sealers' properties. This systematic review compared the physicochemical and biological properties of calcium silicate–based sealers with those of conventional sealers.

Bioceramic-based materials have been recently introduced as root repair cements (1, 2) and root canal sealers (3, 4). Bioceramic products may include alumina and zirconia particles, bioactive glass, calcium silicates, hydroxyapatite, and resorbable calcium phosphates in their formulation (5). In general, these materials are biocompatible, nontoxic, non-shrinking, and chemically stable within the biological environment (4, 6, 7). They also have the ability to form hydroxyapatite during the setting process and ultimately create a bond between dentin and the filling material (3, 4).

There are 2 premixed calcium silicate–based sealers with similar chemical composition, iRoot SP (Innovative Bioceramics, Vancouver, BC, Canada) and EndoSequence BC (Brasseler USA, Savannah, GA). In addition to antibacterial activity (8, 9), they have shown cytocompatibility (6), good sealing ability (3), and good bonding to root canal dentin even under various conditions of dentin moisture (10, 11).

Mineral trioxide aggregate (MTA) Fillapex (Angelus, Londrina, PR, Brazil) has been denominated a bioaggregate (12) or bioceramic-based sealer (13). However, it is a calcium silicate–containing endodontic sealer that is based on salicylate resin and other resinous components (14). MTA Fillapex has alkaline pH and antibacterial activity (15), but it has demonstrated irritating effects on subcutaneous connective tissue (16) and bone tissue (17). Thus, despite the presence of MTA, this material may not have biological advantages.

The epoxy resin–based sealer AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is the gold standard sealer regarding physical properties, and it has shown higher bond strengths to dentin than other root canal sealers (18). AH Plus has been widely used for approximately 2 decades, exhibiting low solubility and disintegration (19) as well as adequate dimensional stability (7). However, this sealer has shown no bioactive properties (14) or osteogenic potential (20).

Premixed calcium silicate–based endodontic products have been introduced to the market for their biological advantages, mainly their bioactivity potential (21, 22). However, up to now, there are few independent publications about their laboratory properties and no long-term clinical trials. In this context, the aim of this study was to compare the physicochemical and biological properties of these relatively novel

From the *Graduate Program in Dentistry, Federal University of Pelotas, Pelotas, Brazil; and †Department of Stomatology, School of Dentistry, Federal University of Santa Maria, Santa Maria, Brazil.

Address requests for reprints to Dr Fernanda Geraldo Pappen, School of Dentistry, Federal University of Pelotas, Rua Gonçalves Chaves 457, Room 507, Pelotas, RS, Brazil 96015-560. E-mail address: ferpappen@gmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.11.019

root canal sealers with those of other conventional sealers by systematically reviewing *in vitro* and *in vivo* animal studies in the literature.

Materials and Methods

This systematic review was carried out according to the guidelines of Cochrane Handbook for Systematic Reviews of Interventions (23), following the 4-phase flow diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (24). This report is based on the PRISMA statement. Despite being a systematic review that is based on laboratory studies, the question of research was adapted from the PICO framework: Population – specimens or animals from *in vitro* and *in vivo* animal studies; Intervention and Comparison – use of premixed calcium silicate–based endodontic sealers versus conventional sealers; Outcome – chemical, physical, or biological properties.

Study Selection and Search Strategy

Medline via PubMed, Scopus, and Web of Science databases were searched. The inclusion criteria were *in vitro* or *in vivo* animal studies that compared the properties of premixed calcium silicate–based endodontic sealers (bioceramic sealers) with those of conventional sealers. Only EndoSequence BC and iRoot SP were considered in the scope of this study because they are premixed materials mainly composed of calcium silicate with potential bioactivity. Non-premixed sealers with different compositions were considered conventional sealers. To be included in this review, the article should have reported at least 1 comparison of specific chemical, physical, or biological characteristics between at least 1 premixed calcium silicate–based endodontic sealer and 1 conventional material, irrespective of the method of analysis. The following properties of interest were considered: bond strength to root dentin, radiopacity, pH, solubility, setting and working time, dimensional change, flow, Ca^{+2} release, antimicrobial activity, biocompatibility, or cytotoxicity. The exclusion criteria comprised articles that evaluated other properties of calcium silicate–based endodontic sealers (eg, sealing ability), articles that tested other bioceramic materials than the target sealers (EndoSequence BC/iRoot SP), or when no comparison between bioceramic and conventional sealers was present.

Date limit was set from 2009, when these specific premixed calcium silicate–based endodontic sealers were developed, to 2016. The last search was carried out in June 2016 with no language restriction. The references of all eligible articles were also hand-searched. A wide search strategy was used to avoid missing information: ("endodontic sealer" OR "root canal sealer"). Literature search results were de-duplicated by using EndNote X7 software (Thomson Reuters, New York, NY). Two independent reviewers (L.H.S.A., R.D.M.) initially screened the titles of all identified studies. If the title indicated possible inclusion, the abstract was carefully appraised, and the articles considered eligible for the review (or in case of doubt) were selected for full-text reading. Discrepancies were resolved by discussion with a third reviewer (F.G.P.)

Data Collection and Analysis

A standardized outline was used for data extraction that was based on the characteristics of the studies and groups tested. Articles were grouped according to the tested property, and the following items were registered: sample size, method of analysis, results (means and standard deviations), and conclusions. The authors were contacted in case of any missing or unpublished data; these studies were only included if the missing information was provided. Considerable heterogeneity was present in the selected studies regarding the research design, methods, outcome variables, and data variability. Because meta-analysis was considered inappropriate, the characteristics of studies were summarized descriptively.

Results

The flowchart of the systematic review is shown in Figure 1. The screening of titles and abstracts initially resulted in 31 articles, and 1 additional article was found by hand-searching. The studies comparing the target sealers only with root repair cements were excluded in this stage. Five articles were excluded after full-text reading because 2 studies did not sufficiently describe their statistical tests or findings (11, 25), 1 study did not compare the sealers (26), and 2 studies used other bioceramic materials than the target sealers (27, 28).

In total, 27 studies were included in this review and processed for data extraction. Supplemental Table 1 shows, in alphabetical order, the commercial name and chemical composition of the materials used in the included studies in comparison with the target calcium silicate–based endodontic sealers (EndoSequence BC/iRoot SP).

Physical-Chemical Properties

Data for the physical-chemical properties are shown in Table 1. Nine studies on bond strength were included (13, 18, 29–35). In comparison with AH Plus, bioceramic sealers showed similar bond strength values in 6 studies (18, 29–32, 34), higher values in 2 studies (33, 35), and lower values in only 1 study (13).

Two studies on radiopacity were included (36, 37), and all tested materials, including EndoSequence BC, exhibited radiopacity higher than the 3-mm aluminum thickness as requested by ISO 6876:2012 (38). With regard to pH values, the bioceramic sealers presented higher pH values than the conventional materials in the 3 included studies (7, 8, 36).

Three studies on solubility were included (7, 10, 14). In 2 studies (7, 10), the bioceramic sealers met the American National Standards Institute/American Dental Association (ANSI/ADA) requirements (39) for solubility (<3%), with similar or higher percentages than AH Plus but lower than Sealapex. In contrast, in the third study (14), iRoot SP did not fulfill ANSI/ADA recommendations, and AH Plus or MTA Fillapex also did not.

Only 1 study was included for working time, setting time, and dimensional change (7). EndoSequence BC had the highest working time and lower values of setting time than other sealers but higher than GuttaFlow. The bioceramic sealer showed slight expansion in accordance with ISO 6876:2012 (38).

Two studies on sealer flow were included (7, 36). In both, the bioceramic sealer (EndoSequence BC) was in conformity with ISO 6876:2012 recommendations (38). Its values were higher than most of the conventional materials (eg, AH Plus) but lower than MTA Fillapex. Concerning Ca^{+2} release, 2 studies were included (14, 36); the bioceramic sealers (EndoSequence BC/iRoot SP) showed higher levels of Ca^{2+} release, when compared with other sealers.

Biological Properties

Data for the biological properties are shown in Table 2. Five studies on antimicrobial activity were included (8, 9, 33, 40, 41). One of these studies used a direct contact test (DCT) against *Candida albicans* and suggested that the bioceramic sealer (iRoot SP) exhibits antifungal activity (41) because it is effective in its freshly mixed form. However, AH Plus showed the highest antifungal effect. In a study using the DCT against *Enterococcus faecalis*, the bioceramic sealer (EndoSequence BC) showed similar antibacterial effect of AH Plus (33).

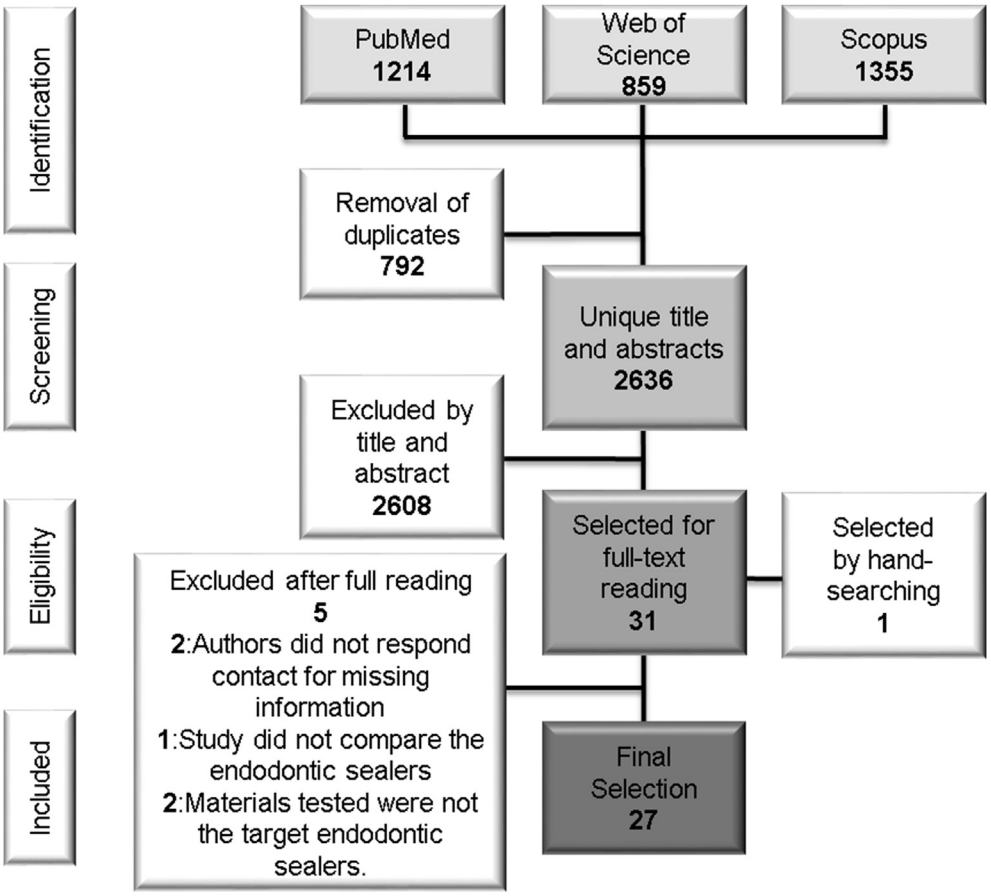

Figure 1. Flow diagram of the study according to the PRISMA statement.

The main findings from the study by Zhang et al (8) indicate that the bioceramic sealer (iRoot SP) kills *E. faecalis* effectively in its fresh form in a DCT test, but the antimicrobial effect was greatly diminished at 7 days after mixing. The same was observed for AH Plus at 1 day after manipulation, whereas Sealapex and EndoREZ maintained their antimicrobial activity throughout the experiment.

Another study used confocal laser scanning microscopy (CLSM). The results showed the bioceramic sealer (EndoSequence BC) had antibacterial effects against *E. faecalis* biofilms within dentinal tubules, similar to AH Plus (9). The study by Willershausen et al (40) evaluated the capacity of different sealers to inhibit *E. faecalis* and *Parvimonas micra* growth by means of scanning electron microscopy (SEM). EndoSequence BC did not inhibit bacteria growth. In general, the bioceramic sealers had superior or similar antibacterial effects when compared with conventional sealers except in 1 study (40).

Regarding biocompatibility, only 1 study, which used subcutaneous connective tissue reaction of Wistar rats, was included (12). The bioceramic sealer (iRoot SP) had absent to mild inflammatory reactions after 90 days. On the contrary, MTA Fillapex remained toxic to subcutaneous tissue.

Nine articles were included on cytotoxicity, and 7 studies used MTT or MTS colorimetric assays with different cell lines (4, 6, 42–46). In general, EndoSequence BC/iRoot SP extracts showed no or low cytotoxicity, with favorable results in comparison with zinc oxide eugenol (ZOE)-based or epoxy resin–based sealers. However, according to Loushine et al (4), EndoSequence BC exhibited severe cytotoxicity on MC3T3-E1 mouse osteoblasts at 24 hours and remained moderately cytotoxic during a 6-week period.

The results of Güven et al (44) showed iRoot SP has less toxic effects than MTA Fillapex on human tooth germ stem cells and may promote better attachment to these cells, as observed under SEM. Willershausen et al (40) used human periodontal ligament fibroblasts and the cell fluorescence method. After 72 and 96 hours, EndoSequence BC showed relatively non-cytotoxic reactions, whereas other sealers caused a significant decrease of cell proliferation. A last study used human gingival fibroblasts and flow cytometry (47), confirming the low cytotoxicity of EndoSequence BC. In general, the bioceramic sealers had similar or better behavior than conventional sealers.

The complete results, found in the systematic review, regarding the physical-chemical and biological properties of premixed calcium silicate–based endodontic sealers in comparison with others are described in Supplemental Tables 2 and 3.

Discussion

To the extent of the authors' knowledge, this systematic review is the first to present a global comparison of physicochemical and biological properties between premixed calcium silicate–based endodontic sealers and conventional root canal filling materials. The target premixed calcium silicate–based sealers are considered bioceramic materials, which have been introduced in the dental practice mainly because of their biocompatibility (12), antibacterial activity (8), and osteogenic potential (45). In this review, the target materials showed good performance, with similar or better results than other commonly used endodontic sealers. Although the present results are based on *in vitro* and *in vivo* animal studies, they provide evidence that may prepare

TABLE 1. Articles Included in the Systematic Review and Overall Findings: Physical-Chemical Properties of Calcium Silicate–based Endodontic Sealers

Property	Author, year	Method	Material	Conclusion
Bond strength	Ersahan and Aydin, 2010 (18)	Push-out test	iRoot SP AH Plus EndoREZ Sealapex	iRoot SP results were similar to AH Plus and superior to EndoREZ and Sealapex
	Sagsen et al, 2011 (31)	Push-out test	iRoot SP AH Plus MTA Fillapex	iRoot SP results were similar to the other sealers in the coronal root third, whereas in the middle and apical segments iRoot SP results were similar to AH Plus and higher than MTA Fillapex
	Amim et al, 2012 (13)	Push-out test	iRoot SP MTA Fillapex AH Plus	iRoot SP results were lower than AH Plus and similar to MTA Fillapex, but when calcium hydroxide or passive ultrasonic irrigation was applied, iRoot SP results were similar to AH Plus and higher than MTA Fillapex
	Shokouhinejad et al, 2013 (32)	Push-out test	EndoSequence BV AH Plus	EndoSequence BC results were similar to AH Plus with or without the smear layer
	Nagas et al, 2014 (30)	Push-out test	iRoot SP RealSeal SE + Resilon AH Plus MTA Fillapex	iRoot SP showed higher resistance to dislocation in the bulk-filled form than the conjunction with the tested core filling materials.
	Tasdemir et al, 2014 (33)	Push-out test	iRoot SP MTA Fillapex	iRoot SP results were greater than MTA Fillapex
	Gade et al, 2015 (35)	Push-out test	EndoSequence BC AH Plus Endomethasone	EndoSequence BC results were higher than AH Plus and Endomethasone when the thermoplasticized technique was used but lower than AH Plus in the lateral condensation group
	Tuncer et al, 2015 (29)	Push-out test	iRoot SP AH Plus MTA Fillapex	iRoot SP results were similar to AH Plus and higher than MTA Fillapex in all root thirds
	Tuncel et al, 2015 (34)	Push-out test	iRoot SP AH Plus	iRoot SP yielded significantly higher push-out strength values than AH Plus
Radiopacity	Candeiro et al, 2012 (36)	Digitized conventional radiographs	EndoSequence BC AH Plus	EndoSequence BC showed lower radiopacity than AH Plus
	Xuereb et al, 2015 (37)	Digital radiographs (PSP plate system)	EndoSequence BC MTA Fillapex Septodont Sealer Apexit Plus	EndoSequence BC showed the highest radiopacity
pH	Zhang et al, 2009 (8)	pH meter	iRoot SP Apexit Plus Sealapex AH Plus Tubli Seal Epiphany non–light-cured EndoRez non–light-cured	iRoot SP showed the highest pH value in all experimental periods, up to 10 days
	Candeiro et al, 2012 (36)	pH meter	EndoSequence BC AH Plus	EndoSequence BC had higher pH than AH Plus. EndoSequence BC presented alkaline pH in all experimental times
	Zhou et al, 2013 (7)	pH meter	EndoSequence BC AH Plus MTA Fillapex ThermaSeal GuttaFlow Pulp Canal Sealer	Endosequence BC sealer presented the highest pH in all experimental times

(*continued*)

TABLE 1. (*continued*)

Property	Author, year	Method	Material	Conclusion
Solubility	Borges et al, 2012 (14)	Water soaking/ANSI /ADA	iRoot SP AH Plus Sealapex MTA Fillapex	iRoot SP showed the highest value of solubility and did not meet ANSI/ADA's requirements
	Ersahan and Aydin, 2013 (10)	Water soaking/ANSI/ ADA	iRoot SP AH Plus Sealapex EndoREZ	iRoot SP results were similar to EndoREZ and AH Plus and lower than Sealapex. iRoot SP met the ANSI/ADA's requirements for solubility
	Zhou et al, 2013 (7)	Solubility testing	Endosequence BC MTA Fillapex Pulp Canal Sealer GuttaFlow AH Plus ThermaSeal	iRoot SP showed the highest value of solubility among the tested materials. iRoot SP showed values in accordance with ISO 6876:2001
Setting time	Zhou et al, 2013 (7)	Setting time	EndoSequence BC AH Plus MTA Fillapex ThermaSeal GuttaFlow Pulp Canal Sealer	EndoSequence BC results were similar to MTA Fillapex; lower than AH Plus, ThermaSeal, and Pulp Canal Sealer; and higher than GuttaFlow
Working time	Zhou et al, 2013 (7)	ISO standard	EndoSequence BC AH Plus MTA Fillapex ThermaSeal GuttaFlow Pulp Canal Sealer	EndoSequence BC had higher working time than the other materials
Dimensional change	Zhou et al, 2013 (7)	Dimensional change	EndoSequence BC AH Plus MTA Fillapex ThermaSeal GuttaFlow Pulp Canal Sealer	EndoSequence BC showed acceptable dimensional change
Flow	Candeiro et al, 2012 (36)	Flow	EndoSequence BC AH Plus	EndoSequence BC flow was higher than AH Plus
	Zhou et al, 2013 (7)		EndoSequence BC AH Plus MTA Fillapex ThermaSeal GuttaFlow Pulp Canal Sealer	EndoSequence BC showed acceptable flow rate, higher than other sealers, except for Pulp Canal Sealer (similar) and MTA Fillapex (lower)
Ca ion release	Borges et al, 2012 (14)	Atomic absorption spectrophotometry	iRoot SP AH Plus MTA Fillapex Sealapex	iRoot showed higher levels of Ca^{2+} release than Sealapex and AH Plus and similar to MTA Fillapex
	Candeiro et al, 2012 (36)	Atomic absorption spectrophotometry	EndoSequence BC AH Plus Control	EndoSequence BC presented greater release of Ca^{2+} than AH Plus, at different periods, up to 10 days

Complete table is available in Supplemental Material.

the ground for clinical studies and/or protocols. Laboratory studies are generally considered of low clinical relevance, but it is clear that their results are useful in the pre-clinical evaluation of new materials and to guide protocols for several clinical approaches, especially considering the absence of evidence from well-designed clinical trials in dentistry (48) and particularly in endodontics (49).

Studies about sealing ability, ie, leakage evaluation, were eliminated during the search process because of the lack of technique standardization, doubtful reliability, and subsequently low impact results (50, 51). In contrast, push-out bond strength studies were included here. This method is based on the shear stress at the interface between dentin and sealer, which is comparable with stresses under clinical conditions (52). In general, the push-out bond strength values for bioceramic sealers were similar or higher than conventional sealers, regardless of the canal moisture condition or presence of smear layer. In most studies, they were equivalent to AH Plus, which is known by its excellent bonding properties. The adequate performance of the target sealers may be related to their self-adhesive nature, which forms a chemical bond to dentin by production of hydroxyapatite during setting (5). Discrepancies among studies could be explained on the basis of differences in experimental designs, including variations on irrigating solutions and obturation technique.

The values of radiopacity obtained for the premixed calcium silicate–based sealers were clinically acceptable (38). However, they were discrepant (3.83 versus 10.80) in the 2 articles included in this review, despite the similarity between specimen sizes in both investigations. One study used conventional occlusal films followed by digitization (36), whereas the other (37) used a digital system based on photostimulable phosphor plates. This fact may have contributed to those differences. AH Plus is known for its

TABLE 2. Articles Included in the Systematic Review and Overall Findings: Biological Properties of Calcium Silicate–based Endodontic Sealers

Property	Author, year	Method	Material	Conclusion
Antibacterial effect	Zhang et al, 2009 (8)	Modified DCT *E. faecalis* (VP3-181)	iRoot SP AH Plus Apexit Plus Tubli Seal Sealapex Epiphany SE EndoREZ	Fresh iRoot SP, AH Plus, and EndoRez killed *E. faecalis* effectively
	Willershausen et al, 2011 (40)	SEM *E. faecalis* (DSM 20478)	EndoSequence BC GuttaFlow Pulp Canal Sealer EWT AH Plus	EndoSequence BC had no antibacterial effect against *E. faecalis,* similar to other sealers
	Ozcan et al, 2013 (41)	DCT *C. albicans* (ATCC 10231)	iRoot SP MTA Fillapex AH Plus GuttaFlow	iRoot SP and MTA Fillapex were equally effective in reducing the number of viable *C. albicans* with lower antifungal activity than AH Plus. All sealers, except GuttaFlow, exhibited antifungal activity when freshly mixed
	Wang et al, 2014 (9)	CLSM *E. faecalis* (VP3-181)	EndoSequence BC AH Plus Pulp Canal Sealer EWT Gutta-percha Sterile water	EndoSequence BC and AH Plus had superior antibacterial effects against *E. faecalis* biofilms within dentinal tubules compared with Pulp Canal Sealer EWT
	Candeiro et al, 2015 (46)	DCT *E. faecalis* (ATCC 29212)	EndoSequence BC AH Plus Control	EndoSequence BC showed similar antibacterial effect against *E. faecalis* in comparison with AH Plus
Biocompatibility	Bósio et al, 2014 (12)	Subcutaneous connective tissue reaction (Wistar rats)	iRoot SP MTA Fillapex DiaRoot Bioaggregate (BA)	iRoot SP and BA were considered biologically acceptable. MTA Fillapex remained toxic to subcutaneous tissue even after 90 days
Cytocompatibility (cell viability)	Zhang et al, 2010 (42)	MTT assay – MG63 cells	iRoot SP AH Plus	iRoot SP and AH Plus were non-cytotoxic in one half and one fourth dilutions
	Zhang et al, 2010 (6)	MTT assay – L929 mouse fibroblasts	iRoot SP AH Plus	iRoot SP was significantly less toxic than AH Plus.
	Loushine et al, 2011 (4)	MTT assay – MC3T3-E1 mouse osteoblasts	EndoSequence BC, fresh EndoSequence BC, set AH Plus, fresh AH Plus, set Pulp Canal Sealer, fresh Pulp Canal Sealer, set	EndoSequence BC showed moderately cytotoxic during the 6-week period
	Willershausen et al, 2011 (40)	Cell fluorescence – human periodontal ligament fibroblasts	EndoSequence BC AH Plus GuttaFlow Pulp Canal Sealer	EndoSequence BC can be considered a biocompatible material. AH Plus and Pulp Canal Sealer showed lower biocompatibility compared with EndoSequence BC and GuttaFlow
	Zoufan et al, 2011 (43)	MTT assay – L929 mouse fibroblasts	EndoSequence BC, fresh EndoSequence BC, set AH Plus, fresh AH Plus, set GuttaFlow, fresh GuttaFlow, set Tubli Seal, fresh Tubli Seal, set	EndoSequence BC and GuttaFlow had lower cytotoxicity than AH Plus and Tubli Seal.
	Güven et al, 2013 (22)	MTS assay – human tooth germ stem cells	iRoot SP MTA Fillapex AH Plus	iRoot SP and AH Plus had lower cytotoxicity than MTA Fillapex. iRoot SP may promote better attachment to human tooth germ stem cells
	Chang et al, 2014 (45)	MTT assay – human periodontal ligament cells	iRoot SP MTA Fillapex Sealapex Apatite Root Sealer	iRoot SP, MTA Fillapex, and Apatite Root Sealer induced superior osteoblastic differentiation and less

(*continued*)

TABLE 2. (*continued*)

Property	Author, year	Method	Material	Conclusion
				inflammatory response than Sealapex in periodontal ligament cells via integrin, mitogen-activated protein kinase, and nuclear factor kappa B signaling pathways
	Candeiro et al, 2015 (46)	MTT assay – human gingival fibroblasts	EndoSequence BC AH Plus Control	EndoSequence BC showed less cytotoxicity than AH Plus
	Zhou et al, 2015 (47)	Flow cytometry – human gingival fibroblasts	EndoSequence BC MTA Fillapex AH Plus	EndoSequence BC exhibited lower cytotoxicity than MTA Fillapex. AH Plus was cytotoxic only as freshly mixed sealer

Complete table is available in Supplemental Material.

outstanding radiopacity and showed 6.93 mm Al average radiopacity (36). Thus, the value reported for EndoSequence BC sealer by Xuereb et al (37), 10.80 mm Al, would not be as beneficial because it could obscure gaps within the obturation (53).

Two studies included in the present review showed solubility values of 0.90% (10) to 2.9% (7) for the target sealers, in agreement with ANSI/ADA and ISO 6876/2012 (38) specifications. Borges et al (14) found 20.64% average solubility for iRoot SP. Excessive values were also detected for MTA Fillapex and Sealapex, whereas AH Plus had the least weight loss in the solubility test (<3%). In this context, some differences in the solubility test may be seen in those studies. Borges et al (14) applied a previously proposed modification (54), aiming to achieve similar results with a decrease in the material volume necessary for the production of samples. Also, the period of time that the sealers remained in the incubator varied from 50% longer than (7) to 3 times the setting time (10, 14). This discrepancy of results warrants further investigation because it could be related to the methodology used.

As mentioned by Ersahan and Aydin (18), iRoot SP is composed of fine hydrophilic particles, which, in conjunction with its active calcium hydroxide diffusion, might explain the highest solubility and Ca^{+2} release reported by Borges et al (14). The extremely small particle size of iRoot SP would elevate the solubility because of the increasing surface area, which would allow more liquid molecules to come into contact to the sealer during the test. The high solubility of iRoot SP was confirmed by SEM analysis that revealed an irregular external surface with increased roughness after the solubility test (14).

EndoSequence BC showed lower values of setting time (2.7 hours) than other materials. Bioceramic sealers need moisture during the setting process. Therefore, a Paris plaster mold was used for this sealer and stored at 37°C and >95% relative humidity for 24 hours before testing. The setting time date from Loushine et al (4) was not included because it did not compare EndoSequence BC sealer with other material. However, they observed some interesting and controversial findings. In the absence of water, the specimens required 72 hours (stored in 100% relative humidity) to achieve the initial set and 240 hours to achieve the final set. There appeared to be a tendency for the initial setting time to increase and the final setting time to decrease when crescent amounts of water were included in the sealer.

Flow rate and dimensional change data complied with the requirements of ISO 6876:2012 specification (38) for all tested sealers. In the 2 studies included in this review (7, 36) these values were superior to AH Plus, which is known for its outstanding flow during clinical use. The filling material is supposed to have good ability to penetrate into dentinal tubules and accessory canals, but an excessive flow rate increases the possibility of extrusion beyond the apical foramen, which is a controversial issue in endodontics. Sealer extrusion could injure the periapical tissues because of the cytotoxicity of several sealers, mainly at the initial stage of setting. In this aspect, a sealer with good biocompatibility may be more favorable (7).

The target sealers presented alkaline pH in all experimental times in the 3 studies included herein (7, 8, 36), with higher values than other established sealers. The pH of AH Plus was alkaline in the fresh samples, whereas after setting, its pH was close to neutral (7, 8). Meanwhile, methacrylate-based sealers, such as EndoREZ and Epiphany, showed acidic pH values throughout the study period (8). An alkaline pH may contribute to the biocompatibility and antibacterial ability of the sealer. It has also been found that an alkaline pH of root canal sealers could neutralize the lactic acid from osteoclasts and prevent dissolution of mineralized components of teeth; therefore, alkaline sealers, especially bioceramic-based products, can contribute to hard tissue formation by activating alkaline phosphatase (7, 45). Regarding Ca^{+2} release, the bioceramic sealers (EndoSequence BC/iRoot SP) have shown significantly better results than AH Plus and ZOE-based sealers (14, 36). Ca^{+2} release favors more alkaline pH of the environment, leading to biochemical effects that may culminate in the acceleration of the repair process (55).

The microbiologic studies included in this review used very diverse methods. One study showed efficacy in reducing the number of viable *C. albicans* just for fresh sealers (41). Other authors showed that sealers that were fresh or 3 and 7 days after mixing effectively killed *E. faecalis* (8). Yet, Wang et al (9) observed that the proportion of killed bacteria increased during the 30 days of exposure to the sealers. One of the challenges in endodontic research has been the lack of standardized *in vitro* and *in vivo* protocols for the testing of antimicrobial effect of sealers. Different methods will probably generate different findings. However, in general terms, the target sealers demonstrated good antimicrobial properties.

When the method of evaluation was SEM, the material of interest demonstrated no capacity to inhibit the bacterial growth. SEM qualitatively evaluates the adherence of microorganisms and biofilm formation by an electron beam. This microscopic technique has been used to visualize the amount and distribution of bacteria on the surface of the biofilm; however, it is very debatable for not showing the viability of these bacteria (56).

Concerning biocompatibility and cytotoxicity, it is of utmost importance that endodontic sealers have an acceptable behavior. In general, the target sealers had better biological properties than AH Plus and other commonly used sealers. Moreover, in the study of Chang et al (45), when compared with others, iRoot SP promoted

osteoblastic differentiation of human periodontal ligament cells to a greater extent and increased calcium deposition and mRNA levels of osteoblastic markers. The present findings indicate the bioactive potential of this sealer; however, clinical studies are necessary for confirmation.

Conclusion

Despite the lack of well-designed, long-term clinical trials, the target premixed calcium silicate–based sealers show good physicochemical and biological properties. In general, the results were similar or better than conventional endodontic sealers, as observed in *in vitro* and *in vivo* animal studies included in this systematic review.

Acknowledgments

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

Supplementary Material

Supplementary material associated with this article can be found in the online version at www.jendodon.com (http://dx.doi.org/10.1016/j.joen.2016.11.019).

References

1. Alanezi AZ, Jiang J, Safavi KE, et al. Cytotoxicity evaluation of endosequence root repair material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:122–5.
2. Damas BA, Wheater MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. J Endod 2011;37:372–5.
3. Zhang W, Li Z, Peng B. Assessment of a new root canal sealer's apical sealing ability. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:79–82.
4. Loushine BA, Bryan TE, Looney SW, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. J Endod 2011;37:673–7.
5. Koch K, Brave D. Bioceramic technology: the game changer in endodontics. Endodontic Practice 2009;2:17–21.
6. Zhang W, Li Z, Peng B. *Ex vivo* cytotoxicity of a new calcium silicate-based canal filling material. Int Endod J 2010;43:769–74.
7. Zhou HM, Shen Y, Zheng W, et al. Physical properties of 5 root canal sealers. J Endod 2013;39:1281–6.
8. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod 2009;35:1051–5.
9. Wang Z, Shen Y, Haapasalo M. Dentin extends the antibacterial effect of endodontic sealers against Enterococcus faecalis biofilms. J Endod 2014;40:505–8.
10. Ersahan S, Aydin C. Solubility and apical sealing characteristics of a new calcium silicate-based root canal sealer in comparison to calcium hydroxide-, methacrylate resin- and epoxy resin-based sealers. Acta Odontol Scand 2013;71:857–62.
11. Nagas E, Uyanik MO, Eymirli A, et al. Dentin moisture conditions affect the adhesion of root canal sealers. J Endod 2012;38:240–4.
12. Bosio CC, Felippe GS, Bortoluzzi EA, et al. Subcutaneous connective tissue reactions to iRoot SP, mineral trioxide aggregate (MTA) Fillapex, DiaRoot BioAggregate and MTA. Int Endod J 2014;47:667–74.
13. Amin SA, Seyam RS, El-Samman MA. The effect of prior calcium hydroxide intracanal placement on the bond strength of two calcium silicate-based and an epoxy resin-based endodontic sealer. J Endod 2012;38:696–9.
14. Borges RP, Sousa-Neto MD, Versiani MA, et al. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. Int Endod J 2012;45:419–28.
15. Faria-Junior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J 2013;46:755–62.
16. Tavares CO, Bottcher DE, Assmann E, et al. Tissue reactions to a new mineral trioxide aggregate-containing endodontic sealer. J Endod 2013;39:653–7.
17. Assmann E, Bottcher DE, Hoppe CB, et al. Evaluation of bone tissue response to a sealer containing mineral trioxide aggregate. J Endod 2015;41:62–6.
18. Ersahan S, Aydin C. Dislocation resistance of iRoot SP, a calcium silicate-based sealer, from radicular dentine. J Endod 2010;36:2000–2.
19. Versiani MA, Carvalho-Junior JR, Padilha MI, et al. A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 2006;39:464–71.
20. Kim TG, Lee YH, Lee NH, et al. The antioxidant property of pachymic acid improves bone disturbance against AH plus-induced inflammation in MC-3T3 E1 cells. J Endod 2013;39:461–6.
21. Moinzadeh AT, Aznar Portoles C, Schembri Wismayer P, Camilleri J. Bioactivity potential of EndoSequence BC RRM putty. J Endod 2016;42:615–21.
22. Guven EP, Tasli PN, Yalvac ME, et al. *In vitro* comparison of induction capacity and biomineralization ability of mineral trioxide aggregate and a bioceramic root canal sealer. Int Endod J 2013;46:1173–82.
23. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0. Available at: http://handbook.cochrane.org. Accessed February 8, 2017.
24. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting item for systematic reviews and metaanalyses: the PRISMA statement. PLOS Med 2009;6:1000097.
25. Ozkocak I, Sonat B. Evaluation of effects on the adhesion of various root canal sealers after Er:YAG laser and irrigants are used on the dentin surface. J Endod 2015;41:1331–6.
26. Razmi H, Bolhari B, Karamzadeh Dashti N, Fazlyab M. The effect of canal dryness on bond strength of bioceramic and epoxy-resin sealers after irrigation with sodium hypochlorite or chlorhexidine. Iran Endod J 2016;11:129–33.
27. Aminov L, Vataman M, Maxim DC, et al. Comparative biochemical evaluation of Ca, P and Mg, after subcutaneous implantation of some biomaterials used in endodontic treatment in prosthetics purpose. Rev Materiale Plastice 2014;51:246–51.
28. Zhou Y, Wu C, Xiao Y. The stimulation of proliferation and differentiation of periodontal ligament cells by the ionic products from Ca7Si2P2O16 bioceramics. Acta Biomater 2012;8:2307–16.
29. Tuncer KA, Tuncer S, Selçuk Gökyay S. Correlation between sealer penetration into dentinal tubules and bond strength of two new calcium silicate-based and an epoxy resin-based, endodontic sealer. J Adhes Sci Technol 2014;28:702–10.
30. Nagas E, Cehreli Z, Uyanik MO, Durmaz V. Bond strength of a calcium silicate-based sealer tested in bulk or with different main core materials. Braz Oral Res 2014;28:1–7.
31. Sagsen B, Ustun Y, Demirbuga S, Pala K. Push-out bond strength of two new calcium silicate-based endodontic sealers to root canal dentine. Int Endod J 2011;44:1088–91.
32. Shokouhinejad N, Gorjestani H, Nasseh AA, et al. Push-out bond strength of gutta-percha with a new bioceramic sealer in the presence or absence of smear layer. Aust Endod J 2013;39:102–6.
33. Tasdemir T, Er K, Celik D, et al. Bond strength of calcium silicate-based sealers to dentine dried with different techniques. Med Princ Pract 2014;23:373–6.
34. Tuncel B, Nagas E, Cehreli Z, et al. Effect of endodontic chelating solutions on the bond strength of endodontic sealers. Braz Oral Res 2015;29:1–6.
35. Gade VJ, Belsare LD, Patil S, et al. Evaluation of push-out bond strength of endosequence BC sealer with lateral condensation and thermoplasticized technique: an *in vitro* study. J Conserv Dent 2015;18:124–7.
36. Candeiro GT, Correia FC, Duarte MA, et al. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod 2012;38:842–5.
37. Xuereb M, Vella P, Damidot D, et al. *In situ* assessment of the setting of tricalcium silicate-based sealers using a dentin pressure model. J Endod 2015;41:111–24.
38. International Organization for Standardization. *International Standard ISO 6876:2012: Dental Root Canal Sealing Materials*. Geneva: International Organization for Standardization; 2012.
39. American National Standards/American Dental Association. *ANSI/ADA Specification No. 57: Endodontic Sealing Material*. Chicago: American National Standards/American Dental Association; 2000.
40. Willershausen I, Callaway A, Briseno B, Willershausen B. *In vitro* analysis of the cytotoxicity and the antimicrobial effect of four endodontic sealers. Head Face Med 2011;7:15.
41. Ozcan E, Yula E, Arslanoglu Z, Inci M. Antifungal activity of several root canal sealers against Candida albicans. Acta Odontol Scand 2013;71:1481–5.
42. Zhang W, Li Z, Peng B. Effects of iRoot SP on mineralization-related genes expression in MG63 cells. J Endod 2010;36:1978–82.
43. Zoufan K, Jiang J, Komabayashi T, et al. Cytotoxicity evaluation of Gutta Flow and Endo Sequence BC sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:657–61.
44. Guven EP, Yalvac ME, Kayahan MB, et al. Human tooth germ stem cell response to calcium-silicate based endodontic cements. J Appl Oral Sci 2013;21:351–7.
45. Chang SW, Lee SY, Kang SK, et al. *In vitro* biocompatibility, inflammatory response, and osteogenic potential of 4 root canal sealers: Sealapex, Sankin apatite root sealer, MTA Fillapex, and iRoot SP root canal sealer. J Endod 2014;40:1642–8.
46. Candeiro GT, Moura-Netto C, D'Almeida-Couto RS, et al. Cytotoxicity, genotoxicity and antibacterial effectiveness of a bioceramic endodontic sealer. Int Endod J 2015;49:858–64.
47. Zhou HM, Du TF, Shen Y, et al. *In vitro* cytotoxicity of calcium silicate-containing endodontic sealers. J Endod 2015;41:56–61.
48. Skupien JA, Sarkis-Onofre R, Cenci MS, et al. A systematic review of factors associated with the retention of glass fiber posts. Braz Oral Res 2015;29:1–8.

49. De-Deus G, Canabarro A. Strength of recommendation for single-visit root canal treatment: grading the body of the evidence using a patient-centered approach. Int Endod J 2016 Mar 11; http://dx.doi.org/10.1111/iej.12621 [Epub ahead of print].
50. Wu MK, Wesselink PR. Endodontic leakage studies reconsidered: part I—methodology, application and relevance. Int Endod J 1993;26:37–43.
51. Verissimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. J Oral Sci 2006;48:93–8.
52. Van Meerbeek B, De Munck J, Yoshida Y, et al. Buonocore memorial lecture: adhesion to enamel and dentin—current status and future challenges. Oper Dent 2003; 28:215–35.
53. Ørstavik D. Materials used for root canal obturation: technical, biological and clinical testing. Endod Topics 2005;12:25–38.
54. Carvalho-Junior JR, Correr-Sobrinho L, Correr AB, et al. Solubility and dimensional change after setting of root canal sealers: a proposal for smaller dimensions of test samples. J Endod 2007;33:1110–6.
55. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. J Endod 2010;36: 16–27.
56. Grundling GL, Zechin JG, Jardim WM, et al. Effect of ultrasonics on Enterococcus faecalis biofilm in a bovine tooth model. J Endod 2011;37:1128–33.