DENTAL MATERIALS 35 (2019) 1065–1072

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Esterases affect the physical properties of materials used to seal the endodontic space

M.Q. Marashdeh[a,b], S. Friedman[a], C. Lévesque[a], Y. Finer[a,b,*]

[a] Faculty of Dentistry, University of Toronto, Ontario, Canada
[b] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 6 March 2019
Received in revised form
29 April 2019
Accepted 30 April 2019

Keywords:
Blood esterases
Salivary esterases
Bacterial esterases
Bioceramic sealer
Epoxy resin sealer
Resin composite
Root canal sealer
Dental material physical properties
Dental restoration performance

ABSTRACT

Materials used to seal the endodontic space are subjected to enzymatic degradative activities of body fluids and bacteria.

Objectives. To assess effects of simulated human salivary, blood and bacterial esterases (SHSE) on physical properties of typical restorative material and root canal sealers.

Methods. Specimens of set methacrylate-based resin composite (Bisfil™2B; RC), calcium-silicate sealer (EndoSequence®; BC) or epoxy-resin sealer (AH-Plus®; ER) were tested after up to 28 Days exposure to phosphate buffered saline (PBS) or SHSE, using ANSI/ADA-57:2000 and ISO-6876:2012.

Results. Regardless of media, microhardness increased with time for BC remained unchanged for ER and decreased for RC ($p < 0.05$). SHSE moderated the increase for BC compared to PBS (28.0 ± 4.8 vs. 38.1 ± 7.9 KHN) at 7 Days, and enhanced the decrease for RC at 7 Days (55.6 ± 7.1 vs. 66.3 ± 6.5 KHN) and 28 Days (52.3 ± 9.2 vs. 62.6 ± 8.5 KHN). Compressive strength was enhanced only for BC by either media. BC expanded with time for both incubation conditions; SHSE moderated the expansion compared to PBS at 7 Days (0.026 ± 0.01% vs. 0.049 ± 0.007%). Shrinkage of ER was similar for both incubation media and was lower than that for RC ($p < 0.05$). Shrinkage of RC was enhanced by SHSE compared to PBS at 7 Days (0.5 ± 0.07% vs. 0.38 ± 0.08%). Weight loss was lowest for ER and highest for BC ($p < 0.05$). It was enhanced by SHSE compared to PBS for BC at 28 Days (2.40 ± 0.2 vs. 2.96 ± 0.19 W L%), and for RC at 7 Days (0.54 ± 0.09 vs. 0.80 ± 0.1 W L%).

Significance. Simulated body fluids and bacterial esterases affected the physical properties of test materials, suggesting potential impacts on sealing ability and resistance to bacterial ingress, and to oth strength ultimately affecting their clinical performance.

1. Introduction

Good quality restoration and filling of the endodontic space are both required for favourable treatment outcomes [1,2], as they form a durable seal that prevents microbial ingress from the oral cavity towards the periradicular tissues. Contemporary concepts of endodontic treatment highlight the bonding to root dentine by the restorative materials used to build-up the endodontic access cavity [3] and by the root canal sealers used to seal the interface between root filling core and root

* *Corresponding author at*: Faculty of Dentistry 124 Edward Street Toronto, Ontario, M5G 1G6, Canada.
E-mail address: yoav.finer@dentistry.utoronto.ca (Y. Finer).
https://doi.org/10.1016/j.dental.2019.04.011
0109-5641/

dentine, to fill anatomic irregularities and to entomb remaining bacteria within dentinal tubules [4]. Degradation of these bonds over time may result in bacterial biofilm proliferation into the restorative and endodontic space [5–7].

Methacrylate-based resin composites [8] are frequently favoured for core build-up restoration due to their dentine-bonding properties [9]. The current benchmark for endodontic core restorations are resin composite systems that are bonded to the tooth-dentine structure by either total- etch or self-etch systems [10].

Root canal sealers are required to possess particular properties, among them adaptation or adhesion to canal walls, no shrinkage upon setting and insolubility in tissue fluids [11]. Compliance is routinely tested in accordance with standards set by the American National Standards Institute/American Dental Association (ANSI/ADA specification 57:2000 [R 2012]) [12] and the International Organization for Standardization (ISO- 6876:2012 [13]. Because excessive dimensional changes, especially shrinkage, are not desirable properties for root canal sealers [14], ANSI/ADA standards set limits to their linear shrinkage (1%), expansion (0.1%) and mass loss when set (3%) [12].

A widely used root canal sealer is AH Plus (Dentsply DeTrey, Konstanz, Germany), an epoxy-based resin complying with all ANSI/ADA specifications except for dimensional change [15] and frequently used as a benchmark [16–19]. Although a methacrylate resin-based sealer had been introduced in the mid-1970s showed adverse effects [20], the more recent focus on dentine-bonding has given rise to self-etch methacrylate resin-based sealers [21–23]. A bonded 'monoblock' [24] of sealer, root filling core and root dentine was expected, that would strengthen the roots and prevent bacterial proliferation into filled canals [23]. Subsequent studies have reported that in-situ dentine bonding of self-etch methacrylate resin-based sealers is inconsistent [25], roots are not strengthened [26], bacterial proliferation is not prevented [5] and clinical outcomes are inferior to those achieved with the use of conventional sealers [22].

More recently, calcium silicate cements have been introduced as root canal sealers due to their bioactivity [27] but also their setting expansion in the range of 0.2%–6% [28]. Of these, the 'bioceramic' (BC) sealer (EndoSequence BC Sealer™, Brasseler USA, Savannah, GA) is a biocompatible material that contains calcium phosphate that enables effective adaptation to root dentine by infusion of sealer particles into dentinal tubules and formation of hydroxyapatite [29,30]. Compared to AH Plus and other calcium silicate sealers, BC sealer is less soluble and maintains an alkaline pH for about 4 months [31]. Despite these favourable properties, recently reported clinical outcomes associated with BC sealer were within the range reported in many previous studies that used other types of sealers [32]. BC sealer requires moisture to set, but contamination with blood or saliva might impair its setting [33,34]. Thus, further investigation is warranted into the impacts of exposure to blood or salivary products on the properties of BC sealer.

Core restorations and subjacent root fillings may become exposed to saliva and bacteria if marginal breakdown of the coronal restoration occurs over time [6,7]. Furthermore, root canal sealers may become exposed to body fluids, mainly blood, through the apical foramen or accessory canals [6,7,35,36]. While early exposure to body fluids can critically impair the sealer's setting process [34], late exposure may also alter the sealer's physical properties and impair its sealing ability [37]. Of particular concern in regards to exposure to body fluids are esterases, a group of enzymes that exist in blood, saliva and bacteria [38–41]. Human blood contains cholesterol esterase (CE) and pseudocholine esterase (PCE) [42]. Saliva and bacteria exert CE-like and PCE hydrolase activities [38,39,41]. As well, specific bacterial esterase from cariogenic bacteria, SMU_118c, acts concurrently with salivary or simulated salivary degradative activities to enhance biodegradation of polymers, including methacrylate resins used in dentistry [39,41,43], decreasing their bond to dentine [8] and increasing bacterial biofilm proliferation within resin-dentine interfaces [6,7]. Thus, exposure to body fluids adversely impact the physical properties of coronal restorative materials, creating pathways for bacterial ingress and saliva toward the root canal sealer and impact clinical outcomes [5,7].

The purpose of this study was to investigate the effects of simulated human salivary esterases on set materials representing those typically used for endodontic core restoration and root filling: methacrylate-based resin composite restorative material, an epoxy-based and calcium silicate root canal sealers. Selected properties including microhardness, compressive strength, dimensional stability and solubility, were used as measures of changed physical properties of the test materials which could impact their clinical performance [14].

2. Materials and methods

2.1. Test materials and aging

Testing methodologies followed ANSI/ADA 57:2000 [R 2012] and ISO (6876:2012) standards. Three materials were tested: RC – Bisfil™2B (Bisco Dental Products, Schaumburg, Ill), a methacrylate-based resin composite; BC – EndoSequence BC Sealer™ (Brasseler USA), a calcium silicate sealer, and ER – AH Plus® (Dentsply DeTrey), an epoxy resin-based sealer. All materials were mixed according to manufacterers' instructions and cylindrical specimens with varying dimensions formed for the specific tests described below. For BC, plaster of Paris moulds with a cavity of 10 mm diameter and 1 mm height were used to allow for setting under humid conditions.

Specimens were initially incubated for 72 h at 37 °C and 95–100% humidity to allow complete setting before testing. To simulate esterase activities from body fluids or bacteria, solutions containing simulated human salivary esterase (SHSE) were prepared and replenished as previously described [6–8,39]. Briefly, SHSE was prepared by mixing cholesterol esterase (CE; COE-313, Lot #86621, Toyobo Co. Ltd., Osaka, Japan) and pseudocholinesterase (PCE; C7612-6KU, Lot #078K7015V, Sigma, St. Louis, MO) in phosphate-buffered saline (PBS) to obtain a solution with 16 units/mL and 0.01 units/mL CE and PCE activity, respectively. Based on stability assays and to maintain SHSE activity, media were replaced daily for the first 5 days of incubation, and thereafter the media was replaced every 10 days and replenished every 5 days for the remainder of the incubation period as previously described [8]. Samples incubated in PBS followed the same replacement/replenishment schedule as those incubated in SHSE in order to maintain consistency between experimental groups. SHSE activity level was verified using nitrophenyl butyrate (pNPB) and butyrylthiocholine (BTC) substrates [8]. For all experiments, specimens were either tested without aging (non-incubated), or after incubation for 7- or 28 Days in either PBS or SHSE (37 °C, pH = 7.0).

2.2. *Microhardness test*

Twenty-five cylindrical specimens (diameter 10 mm; height 1 mm for BC, 2 mm for ER and RC) were tested for each material. The lower height of the BC specimens versus ER and RC improves surface area to volume ratio, allowing for improved diffusion of water into the specimens that is necessary for proper setting under the experimental conditions, as determined by testing prototype specimens. After surface wet-polishing with silicon carbide-based sandpapers (600–1200 grit; 3M, St Paul, MN), specimens were incubated with PBS or SHSE as described above. At the end of the assigned incubation period, specimens were removed, washed, dried and immediately subjected to a microhardness test with a Knoop indenter (Tukon 300, Acco Industries Inc., Bridgeport, CT). Three Knoop Hardness Number (KHN) readings were recorded for each specimen under 100 g loads for 30 s, as a measure of surface microhardness.

2.3. *Compressive strength test*

Fifteen cylindrical specimens (diameter 6 mm; height 12 mm) were tested for each material. Specimens were incubated with PBS or SHSE as described above. At the end of the assigned incubation period, specimens were subjected to compressive loading to failure in a universal testing machine (Instron Corp., Norwood, MA) at a constant crosshead speed of 0.5 mm/min. The load-at-failure (P) necessary to fracture the specimen was recorded. The compressive strength (C) was calculated using the following formula: $C = 4P/\pi D^2$ (where D is the diameter of the specimen).

2.4. *Dimensional change test*

Twenty cylindrical specimens (diameter 6 mm; height 12 mm) for each material had the initial height (H_1) measured with a digital caliper (Mitutoyo Digital Caliper, Kawasaki, Japan). Specimens were incubated with PBS or SHSE as described above. At the end of the assigned incubation period, specimens were lightly dried and their final height (H_2) recorded. The percentage of dimensional change was calculated as follows: $DC\% = (H_2 - H_1)/H_1 \times 100$.

2.5. *Solubility test*

Twenty cylindrical specimens (diameter 20 mm; height 1.5 mm) of each material were placed into a desiccant dehumidifier chamber for 1 h to remove any surface moisture. Each specimen was weighed three times (Shimadzu Corporation, Kyoto, Japan) and the average weight (W_1) recorded. Specimens were then incubated in PBS or SHSE as described above. At the end of the assigned incubation period, specimens were lightly washed, dried and placed in a desiccant dehumidifier for 24 h, then weighed three times and the average weight (W_2) recorded. The percentage of weight loss was calculated as follows: $WL\% = (W_1 - W_2)/W_1 \times 100$.

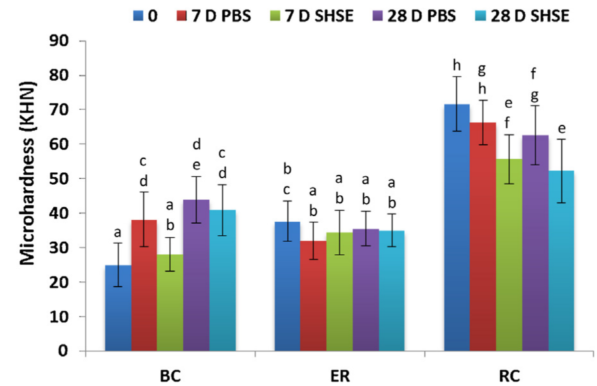

Fig. 1 – Microhardness values (KHN) of the test materials (BC, ER, RC) before and after 7- or 28 Days incubation in either PBS or SHSE. Data are shown as mean ± SD. Statistically significant ($p < 0.05$) differences in microhardness for materials incubated in different media for different periods are identified by different letters.

2.6. *Statistical analysis*

After testing for normal distribution and homogeneity, data were analyzed by three-way analysis of variance (ANOVA). Tukey's post-hoc test ($p < 0.05$) was used to compare the effect of incubation time and conditioning media for the materials, separately for each test: microhardness, compressive strength, dimensional change and solubility.

3. Results

3.1. *Microhardness (Fig. 1)*

RC exhibited the initial highest mean microhardness value (71.7 ± 7.9 KHN), followed by ER (37.7 ± 5.8 KHN) and BC (24.9 ± 6.3 KHN) ($p < 0.001$). After incubation in media, varying patterns of microhardness changes were recorded for the three test materials. For BC, PBS-incubated specimens showed significantly increased KHN values at 7 Days and 28 Days (38.1 ± 7.9 KHN and 43.9 ± 5.2 KHN, respectively; $p < 0.001$). SHSE-incubated BC specimens showed a significant increase only at 28 Days (40.9 ± 7.4 KHN; $p < 0.001$) and the difference between PBS- and SHSE-incubated specimens at 7 Days was significant ($p < 0.005$). For ER, mean KHN values did not vary significantly for the different incubation periods and media. For RC, the initially high mean KHN value (71.7 ± 7.9 KHN) significantly decreased for PBS-incubated specimens at 28 Days (62.6 ± 8.5 KHN; $p < 0.005$). SHSE-incubated specimens showed a significant decrease at 7 Days and 28 Days (55.6 ± 7.1 and 52.3 ± 9.2 KHN, respectively; ($p < 0.005$) compared to the PBS-incubated groups.

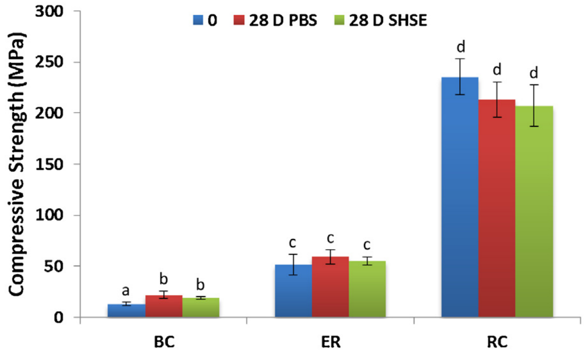

Fig. 2 – Compressive strength values of the test materials (BC, ER, RC) before and after 28 Days incubation in either PBS or SHSE. Data are shown as mean ± SD. Statistically significant ($p < 0.05$) differences in compressive strength for materials incubated in different media for different periods are identified by different letters.

3.2. *Compressive strength (Fig. 2)*

RC exhibited the initial highest mean compressive strength value (235.4 ± 17.6 MPa), followed by ER (51.5 ± 10.1 MPa) and BC (13.1 ± 2.1 MPa) ($p < 0.001$). BC exhibited significantly increased mean compressive strength values after 28 Days incubation in PBS (21.8 ± 13.6 MPa) and in SHSE (19.1 ± 1.5 MPa) ($p < 0.005$). Aging in both media did not affect the compressive strength of both ER and RC.

3.3. *Dimensional change (Fig. 3)*

Varying patterns of expansion and shrinkage were recorded for the three test materials. Initial expansion was recorded for BC at 7 Days for both PBS- and SHSE-incubated specimens (0.05 ± 0.01% and 0.03 ± 0.01%, respectively) that further significantly increased at 28 Days (0.084 ± 0.01% and 0.074 ± 0.01%, respectively; $p < 0.001$). The expansion was significantly higher for PBS than SHSE-incubated BC at 7 Days ($p < 0.001$) but not at 28 Days. For ER, all PBS- and SHSE-incubated specimens exhibited similar shrinkage at 7 Days and 28 Days

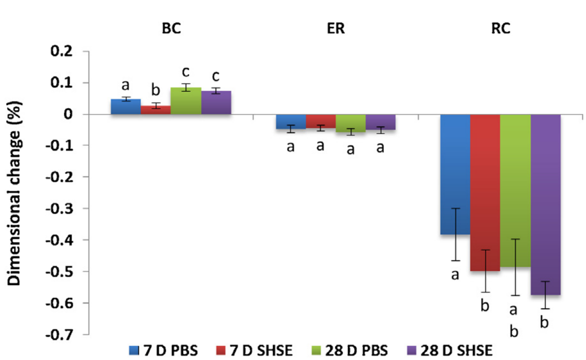

Fig. 3 – Dimensional change of the test materials (BC, ER, RC) after 7- or 28 Days incubation in either PBS or SHSE. Data expressed as percentage of baseline are shown as mean ± SD. Statistically significant ($p < 0.05$) differences within material groups are identified by different letters.

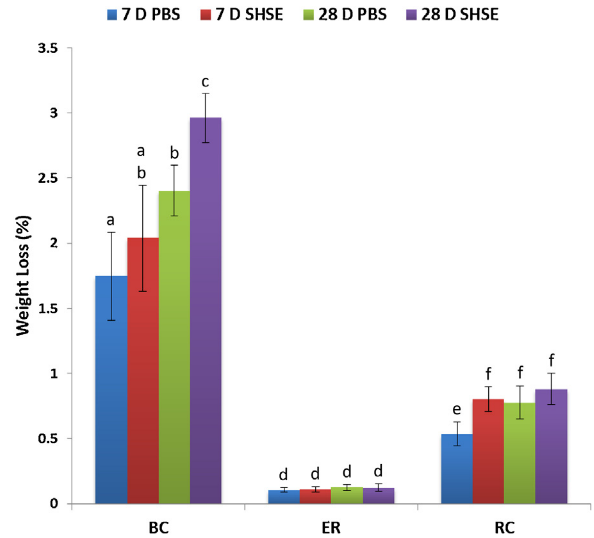

Fig. 4 – Weight loss in the test materials (BC, ER, RC) after 7- or 28 Days incubation in either PBS or SHSE. Data are shown as mean ± SD. Statistically significant ($p < 0.05$) differences in weight loss for materials incubated in different media for different periods are identified by different letters.

($p > 0.05$), with mean values ranging from 0.05 ± 0.01% to 0.06 ± 0.01%. RC exhibited considerable shrinkage with a significantly greater effect of SHSE than PBS at 7 Days (−0.38 ± 0.08% and −0.5 ± 0.07%, respectively; $p < 0.05$) but not at 28 Days (−0.49 ± 0.09% and −0.57 ± 0.04%, respectively). Overall, Shrinkage of RC was significantly higher than that of ER ($p < 0.05$).

3.4. *Solubility (Fig. 4)*

Across the incubation periods and media, BC exhibited significantly higher mean weight loss values (1.75 ± 0.34%–2.96 ± 0.19%), then RC (0.54 ± 0.07% to 0.89 ± 0.03%) and ER (0.11 ± 0.1% to 0.12 ± 0.12%) ($p < 0.01$). For BC, both PBS- and SHSE-incubated specimens showed considerable weight loss that did not differ significantly at 7 Days (1.75 ± 0.34% and 2.04 ± 0.41%, respectively). Weight loss increased significantly at 28 Days for both media ($p < 0.05$) and was significantly higher after incubation in SHSE (2.96 ± 0.19%) compared to PBS (2.40 ± 0.20%) ($p < 0.05$). For ER, all PBS- and SHSE-incubated specimens exhibited initial low weight loss at 7 Days that remained unchanged at 28 Days. For RC, PBS-incubated specimens showed significantly lower weight loss at 7 Days (0.54 ± 0.10%) than SHSE-incubated specimens (0.80 ± 0.10%) ($p < 0.05$). No further significant changes were recorded after 28 Days incubation in both media.

4. Discussion

The physical properties of endodontic restorative materials and root canal sealers impact on their ability to maintain an effective seal of the endodontic space [6–8,11,14,39,44]. Because these materials may become exposed to esterases from tissue fluids and bacteria, this study investigated the impacts of such exposure on the materials' physical properties. The tested materials included a methacrylate-based resin composite, representing common endodontic core build-up materials, as well as two contemporary root canal sealers, an epoxy-resin based sealer (ER) frequently used as standard benchmark [16] and the recently introduced calcium silicate-based sealer (BC) [32].

The materials were tested after aging in buffer or simulated esterases from saliva, blood or bacteria (SHSE), to mimic intraoral physiological and pathological conditions [45]. To our knowledge, the effects of these esterases on root canal sealers are reported herein for the first time. Exposure to PBS established the materials' responses to moisture alone. Aging for up to 28 Days was expected to manifest physical changes in materials [14]. Tests followed ANSI/ADA 57:2000 [R 2012] and ISO 6876:2012 standardized protocols for assessing the physical properties of root filling materials [12,13], and an established methods for aging materials under clinically relevant conditions [6–8,39–41,44–46].

As expected of a typical restorative material, set RC had the highest initial surface microhardness compared to the two root canal sealers. While both media decreased its microhardness, the accelerated and intensified effect by SHSE, reaching the magnitude of 50 KHN, could be attributed to catalyzed hydrolysis of methacrylate-resins by esterase enzymatic activity of blood, saliva and bacteria [38–41]. Softening of RC materials due to water sorption and hydrolysis was shown to reduce their bond strength to dentine [8,44] and sealing ability [6,7], both important parameters for longevity of restorations [47].

The finding that SHSE had minimal or no impact on compressive strength of the test materials was not surprising, since esterases act on surfaces, with limited effect on the bulk of materials [39]. While no minimum compressive strength requirements for root canal sealers have been set, similarity to dentine properties and ability to bond to dentine may be beneficial [24]. Using high strength materials that bond to the tooth structure for both the core and the sealer could enhance tooth rigidity and potentially improve the resistance of filled roots to fracture [24]. In the current study, the compressive strength of RC, comparable to that reported previously and rather similar to that of human dentine (250–350 MPa) [48,49], was not impacted by exposure to either medium.

While polymerization shrinkage of RC is expected upon setting [50], considerable shrinkage of set RC, in the magnitude of 0.5%, occurred after exposure to both media. This effect, accelerated by SHSE, could be explained by the aforementioned hydrolysis and dissolution of unreacted or partially reacted monomeric segments [7,8,39]. Exposure of RC to both media caused weight loss in the magnitude of 0.8%. This mass loss, accelerated initially by SHSE, could be explained by degradation of the ester bonds and release of degradation by-products and dissolution or hydrolysis of unreacted or partially reacted moieties [7,8,39]. Importantly, shrinkage and dissolution of RC have been shown to impair the material's bonding and sealing capacity [5–8,14,44,45]; however, it should be noted that in the current study both effects were within ANSI/ADA limits [12].

BC has been described as a hydrophilic, insoluble cement that can tolerate moisture and small amounts of blood with good antimicrobial, sealing and mechanical properties [29]. In this study, set BC had the lowest initial surface microhardness. While both media increased its microhardness by the magnitude of 16 KHN, this effect was slowed down by SHSE. The hydration process and crystalline structure of BC are affected by environmental conditions ;[34,51,52] the proteins in SHSE could have adsorbed to the surface of the BC material, filling the porosities within the cement thus reducing water uptake and delaying the setting and the hardness increase of BC [51,53,54]. The slight increase in the low initial compressive strength of BC by PBS, as previously reported [55] and SHSE, could be explained by continuing surface hydration and crystallization, reported to continue for up to 4 weeks [56].

Exposure of set BC to both media caused a slight expansion in the magnitude of 0.08%, within the reported limit of 0.2%–6% setting expansion of BC [28] and well within the ANSI/ADA limits [12]. The expansion of BC has been suggested to improve its sealing ability [57]. Nevertheless, contrary to previous reports of minimal solubility [29], BC exposed to both media exhibited considerable weight loss, approaching the ANSI/ADA upper limit of 3% [12]. The enhanced mass loss effect of SHSE could be explained by reduced precipitation reaction and crystallization of the cement due to the aforementioned adsorption of SHSE proteins to its surface that would make it more susceptible to dissolution [51–53]. The increased mass loss of BC when exposed to SHSE might counteract the effect of its expansion and undermine its sealing ability over time.

ER is a widely used root canal sealer and a benchmark in comparative studies of root canal sealers [16–19], due to its favourable properties. In this study, exposure of ER to both media produced comparable effects, with no change in microhardness and compressive strength, as well as minimal shrinkage (magnitude of 0.05%) and weight loss (magnitude of 0.1%). The relative stability of ER, corroborating previous reports [18,19], could be explained by its hydrophobic nature and its composition having no ester bonds.

Excessive dimensional changes are undesirable properties for root canal sealers [14]. Excessive expansion may cause crack initiation or propagation in the root [58]. Excessive shrinkage produces pathways for ingress of bacteria and their products into the endodontic space [6,7,14], a concern that applies also to core restorations [6,7,14]. In this regard, the excellent dimensional stability of ER, characterized by minimal shrinkage, was not adversely impacted by SHSE, supporting its wide use and clinical performance [57]. Similarly, the minor expansion of BC, which may benefit its sealing ability [59], was not hindered by SHSE. In contrast, the considerable shrinkage of RC when exposed to SHSE negated the use of methacrylate-based resins as root canal sealers [5,16]. Indeed, clinical outcomes associated with a methacrylate-resin sealer have not compared well with those reported for other types of sealers [22].

High solubility of root canal sealers is undesirable [60], because dissolution products may act as irritants to periapical tissues [61] and affect bacterial virulence [62,63]. Furthermore, pathways for bacterial ingress may form over time along the sealer-dentine interface [6,7]. The minimal solubility of ER and fairly low solubility of RC were not adversely impacted by SHSE. However, the much higher solubility of BC, while still within the ISO limit, was intensified by SHSE suggesting a potential concern regarding this sealer.

While moisture alone can significantly impact restorative materials and endodontic sealers before and after setting, within the body these materials are exposed to enzymes from blood, saliva and bacteria. Such exposure may occur via interfacial degradation of coronal restorations and core materials [6,7,35,36], the apex and accessory canals, and bacterial biofilms that proliferated within the canal space post-treatment [40,41]. In this study, exposure of the test materials to SHSE was used as a surrogate for such degradative activities. Compared to PBS, this exposure showed inhibition, acceleration or no difference in impacts on the various physical properties of the test materials.

5. Conclusions

All test materials were impacted by exposure to simulated body, bacterial and salivary fluids. While all test materials met the ANSI/ADA 2000 requirements, different impacts of moisture and enzymes on selected physical properties of test materials highlighted the importance of aging in media with enzymatic activities mimicking saliva, blood and bacteria, rather than buffers or water. The relatively high compressive strength of RC was not affected, whereas a decrease in its initially high surface microhardness was enhanced by exposure to enzymes. RC also showed the highest shrinkage and mass loss which are undesirable properties. BC only showed slight increase in surface upper limit solubility that was enhanced by exposure to enzymes. The physical properties of ER were only minimally affected. Extended incubation periods in future studies could reveal longer-term effects of humidity and salivary, blood and bacterial enzymes.

5.1. *Significance*

Simulated body fluids and bacterial esterases affected the physical properties of all tested materials, suggesting potential impacts on sealing ability and resistance to bacterial ingress, and tooth strength ultimately affecting their clinical performance.

Acknowledgments

The authors thank Dr. Mahmood Abu Ruja for his technical assistance and Dr. Jian Wang for assistance with the assessment of the physical properties. This investigation was funded by National Institute of Dental & Craniofacial ResearchR01DE021385; Canadian Institutes of Health ResearchMOP115113; Canada Foundation for Innovation John R. Evans Leaders Fund (CFI_JELF) [project #35378], and Ministry of Research and Innovation (MRI), Ontario Research Fund (ORF) [ORF-35378].

REFERENCES

[1] Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J 1995;28:12–8.
[2] Tronstad L, Asbjornsen K, Doving L, Pedersen I, Eriksen HM. Influence of coronal restorations on the periapical health of endodontically treated teeth. Endod Dent Traumatol 2000;16:218–21.
[3] Taha NA, Maghaireh GA, Ghannam AS, Palamara JE. Effect of bulk-fill base material on fracture strength of root-filled teeth restored with laminate resin composite restorations. J Dent 2017;63:60–4.
[4] Viapiana R, Guerreiro-Tanomaru J, Tanomaru-Filho M, Camilleri J. Interface of dentine to root canal sealers. J Dent 2014;42:336–50.
[5] Roth KA, Friedman S, Levesque CM, Basrani BR, Finer Y. Microbial biofilm proliferation within sealer-root dentin interfaces is affected by sealer type and aging period. J Endod 2012;38:1253–6.
[6] Huang B, Cvitkovitch DG, Santerre JP, Finer Y. Biodegradation of resin-dentin interfaces is dependent on the restorative material, mode of adhesion, esterase or MMP inhibition. Dent Mater 2018;34:1253–62.
[7] Kermanshahi S, Santerre JP, Cvitkovitch DG, Finer Y. Biodegradation of resin-dentin interfaces increases bacterial microleakage. J Dent Res 2010;89:996–1001.
[8] Serkies KB, Garcha R, Tam LE, De Souza GM, Finer Y. Matrix metalloproteinase inhibitor modulates esterase-catalyzed degradation of resin-dentin interfaces. Dent Mater 2016;32:1513–23.
[9] Ilie N, Hickel R. Resin composite restorative materials. Aust Dent J 2011;56(Suppl 1):59–66.
[10] Sofan E, Sofan A, Palaia G, Tenore G, Romeo U, Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type. Ann Stomatol (Roma) 2017;8:1–17.
[11] Grossman LI. Physical properties of root canal cements. 9th ed. Philadelphia: Lea & Febiger; 1976.
[12] ANSI/ADA (2000-R2012). Specification n° 57 Endodontic sealing materials Chicago, USA: ADA Publishing.
[13] ISO-6876. Dentistry — Root canal sealing materials Switzerland the International organization for standardization; 2012.
[14] Orstavik D, Nordahl I, Tibballs JE. Dimensional change following setting of root canal sealer materials. Dent Mater 2001;17:512–9.
[15] Flores DS, Rached Jr FJ, Versiani MA, Guedes DF, Sousa-Neto MD, Pecora JD. Evaluation of physicochemical properties of four root canal sealers. Int Endod J 2011;44:126–35.
[16] Kim YK, Grandini S, Ames JM, Gu LS, Kim SK, Pashley DH, et al. Critical review on methacrylate resin-based root canal sealers. J Endod 2010;36:383–99.
[17] Versiani MA, Carvalho-Junior JR, Padilha MI, Lacey S, Pascon EA, Sousa-Neto MD. A comparative study of physicochemical properties of AH plus and epiphany root canal sealants. Int Endod J 2006;39:464–71.
[18] Schafer E, Zandbiglari T. Solubility of root-canal sealers in water and artificial saliva. Int Endod J 2003;36:660–9.
[19] McMichen FR, Pearson G, Rahbaran S, Gulabivala K. A comparative study of selected physical properties of five root-canal sealers. Int Endod J 2003;36:629–35.
[20] Langeland K, Olsson B, Pascon EA. Biological evaluation of hydron. J Endod 1981;7:196–204.
[21] He J, White RK, White CA, Schweitzer JL, Woodmansey KF. Clinical and patient-centered outcomes of nonsurgical root

canal retreatment in first molars using contemporary techniques. J Endod 2017;43:231–7.
[22] Barborka BJ, Woodmansey KF, Glickman GN, Schneiderman E, He J. Long-term clinical outcome of teeth obturated with resilon. J Endod 2017;43:556–60.
[23] Teixeira FB, Teixeira EC, Thompson JY, Trope M. Fracture resistance of roots endodontically treated with a new resin filling material. J Am Dent Assoc 2004;135:646–52.
[24] Tay FR, Pashley DH. Monoblocks in root canals: a hypothetical or a tangible goal. J Endod 2007;33:391–8.
[25] Tay FR, Hiraishi N, Pashley DH, Loushine RJ, Weller RN, Gillespie WT, et al. Bondability of resilon to a methacrylate-based root canal sealer. J Endod 2006;32:133–7.
[26] Wilkinson KL, Beeson TJ, Kirkpatrick TC. Fracture resistance of simulated immature teeth filled with resilon, gutta-percha, or composite. J Endod 2007;33:480–3.
[27] Gandolfi MG, Taddei P, Tinti A, Prati C. Apatite-forming ability (bioactivity) of ProRoot MTA. Int Endod J 2010;43:917–29.
[28] Prati C, Gandolfi MG. Calcium silicate bioactive cements: biological perspectives and clinical applications. Dent Mater 2015;31:351–70.
[29] Silva Almeida LH, Moraes RR, Morgental RD, Pappen FG. Are premixed calcium silicate-based endodontic sealers comparable to conventional materials? A systematic review of in vitro studies. J Endod 2017;43:527–35.
[30] Al-Haddad A, Che Ab Aziz ZA. Bioceramic-based root canal sealers: a review. Int J Biomater 2016;2016:9753210.
[31] Urban K, Neuhaus J, Donnermeyer D, Schafer E, Dammaschke T. Solubility and pH value of 3 different root canal sealers: a long-term investigation. J Endod 2018.
[32] Chybowski EA, Glickman GN, Patel Y, Fleury A, Solomon E, He J. Clinical outcome of non-surgical root canal treatment using a single-cone technique with endosequence bioceramic sealer: a retrospective analysis. J Endod 2018;44(11):941–5.
[33] Montellano AM, Schwartz SA, Beeson TJ. Contamination of tooth-colored mineral trioxide aggregate used as a root-end filling material: a bacterial leakage study. J Endod 2006;32:452–5.
[34] Nekoofar MH, Oloomi K, Sheykhrezae MS, Tabor R, Stone DF, Dummer PM. An evaluation of the effect of blood and human serum on the surface microhardness and surface microstructure of mineral trioxide aggregate. Int Endod J 2010;43:849–58.
[35] Khayat A, Lee SJ, Torabinejad M. Human saliva penetration of coronally unsealed obturated root canals. J Endod 1993;19:458–61.
[36] Negm MM. The effect of human blood on the sealing ability of root canal sealers: an in vitro study. Oral Surg, Oral Med, Oral Pathol 1989;67:449–52.
[37] Zmener O, Pameijer CH, Serrano SA, Vidueira M, Macchi RL. Significance of moist root canal dentin with the use of methacrylate-based endodontic sealers: an in vitro coronal dye leakage study. J Endod 2008;34:76–9.
[38] Bourbia M, Ma D, Cvitkovitch DG, Santerre JP, Finer Y. Cariogenic bacteria degrade dental resin composites and adhesives. J Dent Res 2013;92:989–94.
[39] Finer Y, Santerre JP. Salivary esterase activity and its association with the biodegradation of dental composites. J Dent Res 2004;83:22–6.
[40] Marashdeh MQ, Gitalis R, Levesque C, Finer Y. Enterococcus faecalis hydrolyzes dental resin composites and adhesives. J Endod 2018;44:609–13.
[41] Huang B, Siqueira WL, Cvitkovitch DG, Finer Y. Esterase from a cariogenic bacterium hydrolyzes dental resins. Acta Biomater 2018;71(a):330–8.
[42] Labow RS, Meek E, Santerre JP. Differential synthesis of cholesterol esterase by monocyte-derived macrophages cultured on poly(ether or ester)-based poly(urethane)s. J Biomed Mater Res 1998;39:469–77.
[43] Huang B, Sadeghinejad L, Adebayo OIA, Ma D, Xiao Y, Siqueira WL, et al. Gene expression and protein synthesis of esterase from streptococcus mutans are affected by biodegradation by-product from methacrylate resin composites and adhesives. Acta Biomater 2018;81:158–68.
[44] Shokati B, Tam LE, Santerre JP, Finer Y. Effect of salivary esterase on the integrity and fracture toughness of the dentin-resin interface. J Biomed Mater Res B, Appl Biomater 2010;94:230–7.
[45] Delaviz Y, Finer Y, Santerre JP. Biodegradation of resin composites and adhesives by oral bacteria and saliva: a rationale for new material designs that consider the clinical environment and treatment challenges. Dent Mater 2014;30:16–32.
[46] Stewart CA, Hong JH, Hatton BD, Finer Y. Responsive antimicrobial dental adhesive based on drug-silica co-assembled particles. Acta Biomater 2018;76:283–94.
[47] Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/dentin interface: the weak link in the composite restoration. Ann Biomed Eng 2010;38:1989–2003.
[48] Jayanthi N, Vinod V. Comparative evaluation of compressive strength and flexural strength of conventional core materials with nanohybrid composite resin core material an in vitro study. J Indian Prosthodont Soc 2013;13:281–9.
[49] Zaytsev D, Grigoriev S, Panfilov P. Deformation behavior of human dentin under uniaxial compression. Int J Biomater 2012;2012:854539.
[50] Pawar SS, Pujar MA, Makandar SD. Evaluation of the apical sealing ability of bioceramic sealer, AH plus & epiphany: an in vitro study. J Conservative Dent: JCD 2014;17: 579–82.
[51] Saghiri MA, Shabani A, Asatourian A, Sheibani N. Storage medium affects the surface porosity of dental cements. J Clin Diagn Res: JCDR 2017;11:ZC116–9.
[52] Ashofteh Yazdi K, Ghabraei S, Bolhari B, Kafili M, Meraji N, Nekoofar MH, et al. Microstructure and chemical analysis of four calcium silicate-based cements in different environmental conditions. Clin Oral Investig 2019;23: 43–52.
[53] Gandolfi MG, Iacono F, Agee K, Siboni F, Tay F, Pashley DH, et al. Setting time and expansion in different soaking media of experimental accelerated calcium-silicate cements and ProRoot MTA. Oral Sur, Oral Med, Oral Pathol, Oral Radiol, Endod 2009;108:e39–45.
[54] Song M, Yue W, Kim S, Kim W, Kim Y, Kim JW, et al. The effect of human blood on the setting and surface micro-hardness of calcium silicate cements. Clin Oral Investig 2016;20:1997–2005.
[55] Guo YJ, Du TF, Li HB, Shen Y, Mobuchon C, Hieawy A, et al. Physical properties and hydration behavior of a fast-setting bioceramic endodontic material. BMC Oral Health 2016;16:23.
[56] Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A. Comparison of physical and mechanical properties of mineral trioxide aggregate and biodentine. Indian J Dent Res 2014;25:692–7.
[57] Li GH, Niu LN, Zhang W, Olsen M, De-Deus G, Eid AA, et al. Ability of new obturation materials to improve the seal of the root canal system: a review. Acta Biomater 2014;10:1050–63.
[58] Islam I, Chng HK, Yap AU. Comparison of the physical and mechanical properties of MTA and portland cement. J Endod 2006;32:193–7.

[59] Camilleri Jv. Evaluation of selected properties of mineral trioxide aggregate sealer cement. J Endod 2009;35:1412–7.
[60] Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. J Endod 2013;39:1281–6.
[61] Cintra LTA, Benetti F, de Azevedo Queiroz IO, Ferreira LL, Massunari L, Bueno CRE, et al. Evaluation of the cytotoxicity and biocompatibility of New resin epoxy-based endodontic sealer containing calcium hydroxide. J Endod 2017;43:2088–92.
[62] Sadeghinejad L, Cvitkovitch DG, Siqueira WL, Merritt J, Santerre JP, Finer Y. Mechanistic, genomic and proteomic study on the effects of BisGMA-derived biodegradation product on cariogenic bacteria. Dent Mater 2017;33:175–90.
[63] Sadeghinejad L, Cvitkovitch DG, Siqueira WL, Santerre JP, Finer Y. Triethylene glycol Up-regulates virulence-associated genes and proteins in streptococcus mutans. PloS One 2016;11:e0165760.