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Physical Properties of New Generation Tricalcium Silicate Dental Materials

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Abstract

Tricalcium silicate-based materials are growing in popularity for dental procedures. This study reports the physical properties of two experimental tricalcium silicate-based Generex A and B materials. Generex A is designed for vital dental pulp therapy, the repair of root perforations, or to seal a resected root apex. Generex B is designed for nonsurgical root canal procedures as a sealant. ADA 57 and ISO 9917 methods were used for testing in vitro properties of flow, working and setting times, film thickness, dimensional stability, solubility, radiopacity, compressive strength, and freedom from lead and arsenic. In vitro tests of fluid flow were conducted to compare microleakage. Generex A met the ADA 57 and ISO 9917 requirements as they apply for the intended use of this material; Generex B met the ADA 57 requirements for a root canal sealer. Both materials had lower film thickness and higher radiopacity, than ProRoot® MTA. The setting times of the Generex materials were no shorter than MTA, but the handling was very much improved over MTA. Furthermore, these materials sealed as well as the standard ProRoot MTA material. These new materials are suitable for testing in animal models for their intended use.

Introduction

Diseased or traumatized require treatment to stimulate healing of the injured dental pulp, or in cases of irreversible pulpal damage, to replace the pulp using nonsurgical root canal treatment. For recalcitrant apical infections, root-end resection surgery is used to seal the root canal.

An experimental, gray-colored, calcium silicate-based material, Mineral Trioxide Aggregate (MTA), was introduced in the 1990s [1] followed by commercialized forms of MTA, ProRoot® MTA and tooth-colored ProRoot MTA, (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), and later, MTA Angelus® and MTA Bianco, (Angelus, Londrina, Brazil). MTA products are used for pulp capping, pulpotomy, apical barrier formation in teeth with immature root formation and open apices, perforative root resorption defects, surgical root-end filling, and repair of iatrogenic root perforations occuring during clinical root canal procedures. Remarkably, MTA stimulates the formation of hydroxyapatite on its surface [2-6] to facilitate healing [7]. Furthermore, MTA has excellent sealing [8,9], preventing bacterial migration into the tooth [10]. MTA-type powders contain mainly tricalcium silicate, dicalcium silicate, and a radiopaque agent, but often include minor amounts of tricalcium aluminate, calcium sulfate, and tetracalcium aluminoferrite [11]. White forms of MTA contain less grey tetracalcium aluminoferrite.

Clinicians have avoided the use of MTA because of its coarse particle size [12], lengthy setting time of several hours [13], high price [14,15], and initial instability in the site of application. The latter results in washout of the material after placement during commonly used irrigation procedures [16]. Ideally, new materials would have better working properties while retaining the biological advantages of MTA.

To counteract these concerns, liquid additives have been tested to shorten the setting and improve working characteristics: chlorhexidine gel, sodium hypochlorite (3%), K-Y™ Jelly, and calcium chloride solutions (3 and 5%) [12]. Calcium chloride solutions accelerate setting of MTA [17]; however, the setting time reported for white MTA mixed with water was only 12 min. This setting time is much shorter than that first reported for the experimental gray MTA [13] (165 min), indicating either technique variations or materials changes. Three percent methylcellulose and calcium chloride in a gel accelerated the development of strength and reduced the setting time of MTA [18]. Disodium hydrogen phosphate (Na_2HPO_4) solutions also accelerated setting to less than 50 minutes [19].

The powder formula has also been altered to include magnesia and zinc oxide, reducing the setting time to less than 15 min. from 151 min., but decreased the strength [20]. Gypsum reduced the setting time to as low as 20 minutes [21]. Camilleri [22] combined calcium aluminate cements with tricalcium silicate cement to achieve faster setting (6 min) with a "superplasticizing" admixture to improve the handling. Tricalcium silicate powder with calcium oxide, calcium phosphate, calcium carbonate, calcium sulfate, calcium hydroxide, and calcium chloride [23] set faster (50 vs. 70 min), had a lower film thickness (174 vs. 452 µm) and higher flow (14 vs. 10 mm) compared to tooth-colored (white) ProRoot MTA. A new generation of tricalcium silicate-based materials with improved handling and faster setting is desired.

The Generex A and B experimental materials tested in the present study are based on tricalcium silicate, and have been evaluated for

their osteogenic potential using primary osteoblasts [24]. The Generex A material, designed for the same indications as MTA, has been tested for some of its in vitro properties [16], and had superior handling and washout resistance compared with tooth-colored ProRoot MTA.

The Generex B experimental material is a root canal sealer (also known as ProRoot Endo Sealer) [25,26] that was reported to form hydroxyapatite in simulated body fluid, and was "minimally tissue irritating" [27]. These outcomes are highly desirable for a material that may be expressed inadvertently beyond the root apex into the bone during root canal obturation procedures [28]. Commonly used zinc oxide-eugenol sealers are cytotoxic and are not favorable to the periapical tissues when expressed past the apical foramen [29].

The purpose of the present study was to measure the in vitro properties of the two experimental Generex materials, to establish their suitability for animal testing and clinical applications using standardized methods for dental materials.

Materials and Methods

Two experimental, tricalcium silicate-based materials, Generex A and Generex B (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), were supplied as two powders, each having its own unique gel. Being based on tricalcium silicate, the same high pH and calcium ion release of other MTAs were characteristic of these material. The Generex A powder was mixed with its gel at 3:1 by weight; the Generex B mixing ratio was 2:1. The sample preparation and test methods used by others [30-32], from the American National Standards Institute ANSI-ADA Specification 57 (2000), entitled "Endodontic Sealing Materials" and the International Standards Organization ISO 9917 (2003), entitled "Water-based cements: Powder/liquid acid-base cements", were adopted to facilitate comparisons to others' measurements for calcium silicate hydrate (MTA-type) materials [23]. The ADA 57 methods are identical to the ISO 6876:2001 dental root canal sealer material standard, except for the size of the flow sample. Samples of the powders and their corresponding gels, were tested for their flow, working time, setting time, dimensional stability, solubility, film thickness, radiopacity, compressive strength and acid-soluble lead and arsenic contents. The testing was performed at Dental Advisors (Ann Arbor,MI USA) with the exception of the radiopacity performed at Baylor College of Dentistry (Dallas, TX USA). Any exceptions to the methods are noted below. A computerized fluid flow apparatus was used for leakage evaluation at Georgia Regents Univeristy, Augusta, GA USA.

Flow

Flow tests were performed by expressing 0.5 mL of mixed material onto a glass plate. The material was applied 3 min after mixing, forming a sandwich with another glass plate. Extra weights, totaling 120 gm, were applied for an additional 7 min. The diameter of the material was measured to indicate flow.

Working time

The flow tests were repeated with a longer interval before application of 120 gm weight onto the 0.5 mL of mixed material sample for successive samples, until the resulting flow diameter was 10% smaller than the flow test.

Setting time

Setting time was determined with a Gilmore needle apparatus using 2 mm thick, 20 mm diameter samples. The Gilmore needle, with an indenter having a flat end of 1.0 mm in diameter, and delivering a 100 g load, was placed on the material periodically. The setting time was determined when the needle no longer produced an indentation, as determined by unaided visual examination. The Generex materials include a water-based gel, which begins to evaporates before setting is complete. Therefore, the specimens were stored in closed Petri dishes in a humidor with a wet towel at 37°C during periods between Gilmore indents. Polyethylene sheets were placed on the top and bottom of the ring mold to reduce evaporation. Experiments were conducted in triplicate; the mean of the three measurements was the setting time.

Other specimens were tested in a similar fashion, but the mixed material was placed in a 20 mm diameter by 1 mm thick cavities in a plaster mold, which had been acclimatized at 37°C and 95% relative humidity for more than 24 h. This cavity diameter was larger than the value specified in ADA 57 or ISO 6876 (10 mm), but was useful for providing the area for repeated testing for materials that set slowly.

Dimensional stability

Dimensional stability was measured using specimens 6 mm in diameter and 12 mm long. The specimens were kept in a humidor for 3 days at 37°C, unmolded and their flat ends ground with sandpaper. A stereomicroscope was used to measure the length of each specimen. Samples were marked and stored in distilled water at 37°C for 30 days, after which their lengths were re-measured. The mean percentage change in length of the 3 specimens was calculated.

Solubility

The solubility test required specimens that were 1.5 mm thick and 20 mm in diameter, set at 37°C. Specimens were placed in a sealed humidor with wet towels for 72, 96 or 120 h unmolded, smoothed to remove flash, weighed. The samples were submerged in a previously weighed Petri dish with 50 mL of distilled water at 37°C for 24 h. Then the samples were removed, but rinsed to return any loose material into the water. The water was slowly evaporated and the residue remaining in the dish was weighed to determine the solubility. The test was performed in triplicate for each setting period.

Film thickness

Film thickness was measured by placing mixed materials between two glass plates. A force of 150 N was applied to the glass plates 2.5 min after initiation of mixing. The thickness of each specimen between the glass plates was measured with a micrometer after a total of 10 min. Measurements were performed in triplicate.

Radiopacity

Radiopacity was determined by placing mixed material in the center of a glass slide. One-millimeter thick spacers were placed on either end of the glass slide and a second glass slide was placed on top. The ends with the spacers were taped to create a stable sandwich as the material set. The sample and an aluminum step-wedge, having 10 mm width and 1 mm high steps, were exposed to a dental X-ray source (Model 1000, GE Healthcare, Waukesha, WI) operating at 60 kV, and 8 mA, with a focal-phosphor plate distance of 10 cm. Visix 2.0.0 (Visix Inc.,

Norcross, GA, USA) software used to read the size 4 phosphor plates (Air Techniques, Inc., Melville, New York, USA). The images were imported into Photoshop® (Adobe®, San Jose, CA, USA) and evaluated to determine the thickness of the aluminum step-wedge that corresponded with the radiographic density of the specimen, by overlaying part of the specimen's image on the step-wedge's image. The ADA 57 or ISO 6876 methods for radiopacity used film, not digital x-rays, but the sample thickness is the same.

Compressive strength

Compressive strengths were measured using a modified version of the ISO 9917 standard [33]. A two-piece Delrin split mold, with an inner diameter of 4 mm and height of 8 mm, was used to make cylindrical samples. The ISO 9917 standard requires testing performed after 24 h., but the MTA samples set at 37°C and 95% humidity for 7 days. After unmolding, each specimen was abraded with 320-grit sandpaper to create flat-ended samples for testing. Eight defect-free samples were compressively loaded to failure, with an Instron® 5866 universal testing machine (Instron Corp., Norwood, MA, USA) along the longitudinal axis of each cylinder, at a crosshead speed of 0.5 mm/min. The load-at-fracture and the precise sample dimensions were used to calculate compressive strength.

Leachable heavy metals

The sample preparation method described in the ISO 9917 standard was used to determine the leachable arsenic and lead. Three grams of the non-radiopaque components of the Generex powders were mixed with water, allowed to set for 24 h at 37°C, and crushed with a mortar and pestle. Two grams of crushed powder were placed in 0.1 N HCl for 16 h. Elemental analysis was performed using inductive coupled plasma mass spectroscopy (Lancaster Laboratories Inc., Lancaster, PA, USA). The total arsenic content and lead contents were determined for the radiopaque components and the set cement, after dissolution in aqua regia using inductively coupled plasma spectroscopic techniques. A deviation was made from the ISO 9917 standard, which refers to wet chemical analysis for arsenic.

Sealing

Two sealing tests were performed with differences in the sample preparation for the two Generex materials. In each test, recently extracted, human, single-rooted teeth were obtained from unknown individuals following guidelines of an MCG-approved Human Assurance protocol (#06-05-396). The teeth were stored in 0.9% NaCl solution containing 0.02% sodium azide at 4°C to prevent bacterial growth until ready for use. All teeth were decoronated at the cementoenamel junction under copious water-cooling, to create root segments that were all approximately 17 mm long. Teeth were randomly distributed into experimental groups (N=12), a positive control group (N=3) and a negative control group (N=3). Cleaning and shaping of the root segments were performed under an operating microscope (Carl Zeiss Surgical, Inc., Thornwood, NY, USA). Six percent sodium hypochlorite was employed, followed by 17% ethylenediamine tetraacetic acid as the final root canal irrigant to remove organic and inorganic debris in the root canal.
For the Generex A test, the apical 3 mm of each root was resected, with a 10° bevel. Root-end preparations were made to a depth of 3 mm using an ultrasonic unit (Satelec P5, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). Three materials were tested: Generex A, White ProRoot® MTA (both from Dentsply Tulsa Dental Specialties), and SuperEBA™ (Bosworth, Skokie, IL, USA). Each material was mixed according to the manufacturer's instructions and placed in the root-end, abutting a gutta-percha cone. Two positive controls were used (N=3) for maximum fluid flow: teeth with empty canals (cleaned/shaped root canals without any root filling material), and teeth with canals containing gutta-percha but no sealer. The negative control (N=3) consisted of cleaned-and-shaped root segments with dental resin composite in the apical end, dipped into molten sticky wax, and covered with varnish. After filling, the teeth were stored at 95% relative humidity at 37°C for 24 h, and then stored in phosphate buffered saline for 3 days before evaluation. After the first fluid filtration testing, the roots were returned to the saline solution, incubated at 37°C for 39 days, and then retested.

The second protocol for the root canal sealers was filling the root canals with gutta percha and the root canal sealer, the usual root canal treatment. All canals were prepared to ISO size 40, 0.06 taper. The working lengths of the extracted teeth were established at 1 mm short of the apex. The experimental sealer Generex B (Dentsply Tulsa Dental Specialties) or the control sealer (Kerr Pulp Canal Sealer, SybronEndo, Orange CA, USA) was applied to a size 40, 0.06 taper gutta-percha master cone, fitted to within 1 mm of the working length and root canal obturation was performed. Subsequently, the teeth were stored at 100% relative humidity at 37°C for 7 days before the first leakage measurement. The sealer samples were returned to 37°C in NaCl/sodium azide solution, for twenty-one more days before a second leakage measurement.

For either protocol, leakage was evaluated using a modified fluid filtration protocol [34]. Briefly, a Plexiglas platform with stainless steel tubing in the center was cemented to a 2 mm deep cavity in the coronal end of each root segment. The Plexiglas-root assembly was attached to a fluid filtration apparatus. A small (2 μL) air bubble was introduced into the system and a pressure of 69 kPa (i.e. 10 psi) of nitrogen gas was applied, which forced the solution through the voids along the root canal filling, and displaced the air bubble whose position was monitored. Three 15-min measurements of the linear movement of the air bubble were made for each root segment, from which the mean linear fluid movement was calculated. The mean linear fluid movement was obtained by multiplying the results with a proportionality constant (25 μL/65 mm =0.386 μL/mm), and expressed in μL min^{-1}.

Each leakage test involved experimental groups at 2 time intervals. Thus, a two-way repeated measures analysis of variance (ANOVA) was applied to examine the effect of "material" and "time interval". The data were not normally distributed (Shapiro-Wilk test), so the hydraulic conductance data were transformed into ranks prior to the analysis. Post-hoc comparisons were performed using the Student-Newman-Keuls test, and significance was set at $\alpha=0.05$. The statistical analysis was complemented with individual pair wise analyses. Two Mann-Whitney rank sum tests were used to examine the differences between the control and the experimental groups at both time intervals. Wilcoxon signed rank tests were used to examine the effect of time on leakage.

Results

The test results are listed in Table 1a for Generex A and B materials, with the ADA 57 or ISO 9917 requirements and results for MTA materials from the literature. Table 1b shows the leakage results. MTA

was used for comparisons because all three are tri-calcium silicate-based materials.

Flow

The flow for the Generex B sealer (29 mm) was more than the 20 mm as required for a root canal sealer, where flowing into complex root canal anatomy and around the gutta-percha filling material is necessary. The Generex A material for root and vital pulp treatment had less flow (17 mm), which is suitable for its use in root-end filling, perforation repair, or vital pulp treatments.

Working time

The working time for the Generex B sealer was 65 min. A root-canal sealer is often used to fill several root canals in multi-rooted molar teeth, and this time was suitable. The Generex A material's working time was shorter (9.5 min), and is suitable for its planned indications.

Setting time

In the solid mold, the Generex setting times were considerably longer than the working times: 9 h for the Generex B sealer, and 2.5-11 h for the Generex A vital pulp and root treatment material, depending on when the plastic cover sheets were removed. For the plaster mold specimens, the setting times for all three materials were longer than 1 h.

Property	ADA 57 Requirement	Generex A		Generex B		White ProRoot MTA (Unless otherwise noted, data from reference 25)
Flow (mm)	>20	16.9 ± 0.7		29.1 ± 0.3		
Working time (min.)	None	9.5		65		5
Setting Time (hours)	None	**STEEL MOLD**	**PLASTER MOLD**	**STEEL MOLD**	**PLASTER MOLD**	1.2
		2.5, uncovered	1:10, >4	9, covered	2:20, >4	PLASTER MOLD* 1:03, 1:40
		7-8.5, uncovered after 1 or 2 hrs.				
		24.5, covered				
Dimensionalstability (%)	-1 to +0.1	-0.4 ± 0.3		-0.02 ± 0.03		
Solubility (Weight %)	<3 .0	2.0 ± 0.2		4.6 ± 0.6 @ 24 hr		
				2.0 ± 0.4 @ 120 hr		
Film hickness (µm)	<50	83 ± 54		32 ± 10		
Radiopacity (Equiv. mm of Al)	>3	7.0 ± 0.5		6 ± 0.5		5*, 3, 6 [37], 7.5 [32]

Table 1a: Physical Properties Comparisons vs. ADA 57/ISO 6876 Criteria

Property	ISO 9917 Requirement	Generex A	Generex B	White ProRoot MTA
Compressive strength @ 7 days (MPa)	>50	69.0 ± 7.3	32.1 ± 2.6	40 ± 4 [13] @ 1 day for gray MTA
				67 ± 7 [13] @ 1 day for gray MTA
Leachable arsenic (ppm)	< 2	0.5	0.6	0.009 [43]
Leachable lead (ppm)	<100	<0.5	<0.5	Not detected

Table 1b: Physical Properties Comparisons vs. ISO 9917 Criteria

Dimensional stability

Both Generex materials had slight shrinkage after 30 days (-0.02 and -0.4% for Generex A and B, respectively). These values conformed to the ISO requirement for dimensional stability, which allows up to 1% shrinkage.

Solubility

The solubility of the Generex A was 2.0%, and is less than the required 3% after 24 h. By contrast, the Generex B sealer's solubility was 4.6% after 24 h, decreasing to 2.0% after 120 h of setting (Figure 1). Interpolation of the measured values indicates that the setting time to achieve less than 3% solubility would be achieved after 92 hours.
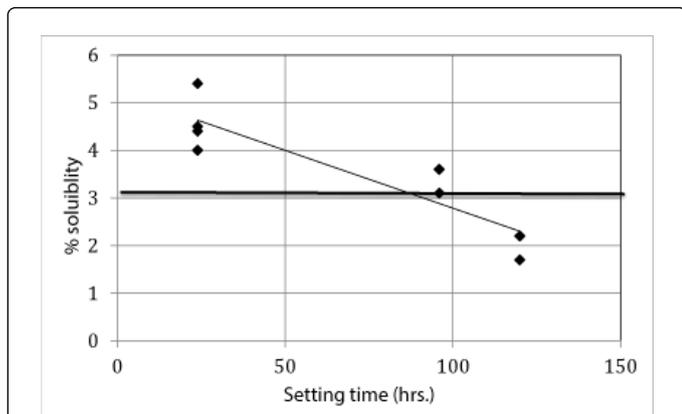
Figure 1: Results of solubility tests depending on curing time for Generex A root canal sealer.

Film thickness

The film thickness requirement of less than 50 μm was met for the Generex B sealer (32 ± 10 μm). Generex A material had a larger and more variable film thickness (83 ± 54 μm).

Radiopacity

The radiopacity of Generex A and B materials exceeded the ISO 6876 requirement of 3 mm equivalent thickness of aluminum, having 7 and 6 mm of radiopacity, respectively, as shown in Figures 2a and 2b for 1-mm thick samples. White MTA contains 20% bismuth oxide by weight [35], and its radiopacity as measured using the present technique was 5 mm.

Compressive strength

The compressive strength of the Generex A vital pulp and root treatment material met the 50 MPa requirement for ISO 9917 water-based dental cements, having 69 MPa compressive strength at 7 days. Generex B sealer fell short of the required strength for water-based dental cements at 32 MPa. However, the ADA 57 or ISO 6876 standards for root canal sealers do not have a strength requirement.

Leachable heavy metals

The non-radiopaque components of the Generex powders exhibited less than 0.92 ppm of leachable arsenic, which meets the ISO 9917 standard. The radiopaque components did not contain detectable arsenic; therefore, the materials met the ISO 9917 standard for leachable As. None of the powder components had detectable lead. The inductive coupled plasma detection limit was 0.49 ppm, which is well below the 100 ppm limit in ISO 9917.

Sealing test

Fluid leakage in the "root-end" leakage test was greatest for the Super EBA group was significantly higher than Generex A or White ProRoot MTA (p<0.001) (Figure 3). No difference was found between the Generex A and the White ProRoot MTA specimens (p>0.05). Fluid leakage during the initial 3-day testing period was significantly higher than the subsequent 42-day testing period (p<0.05) for each material,

and leakage reduction after aging was greatest in the Super EBA group. The negative controls had no leakage. The positive control specimens exhibited a leakage of 105.6 ± 23.2 mL/min, which was 390,000-1,380,000 times higher than the fluid flow than in the experimental groups. When the Super EBA group was excluded, the use of a more robust parametric statistical analysis showed significantly lower leakage in the Generex A for both testing periods (p<0.05) than for White ProRoot MTA.
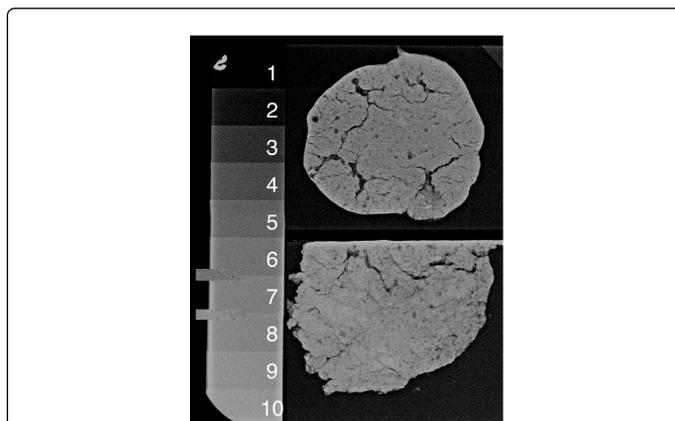

Figure 2a: Radiopacity sample images. The aluminum step wedge is shown on the left . A rectangle, copied from each Generex sample is overlaid on the wedge to illustrate the relative radiopacity (Generex A).
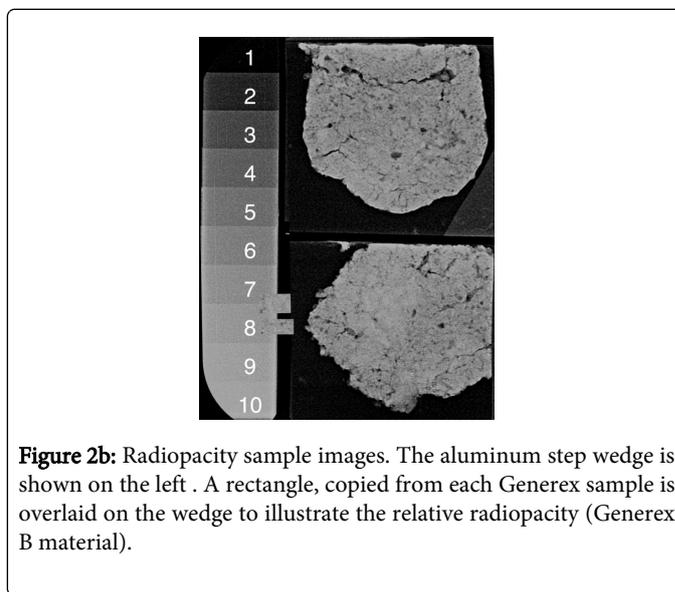

Figure 2b: Radiopacity sample images. The aluminum step wedge is shown on the left . A rectangle, copied from each Generex sample is overlaid on the wedge to illustrate the relative radiopacity (Generex B material).
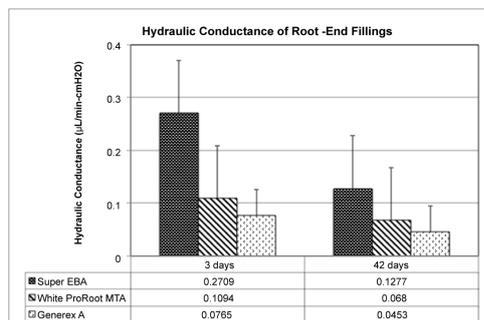
Figure 3a: Hydraulic conductance of materials tested for root end filling. Sealing improved with time and the use of tricalcium silicate materials.
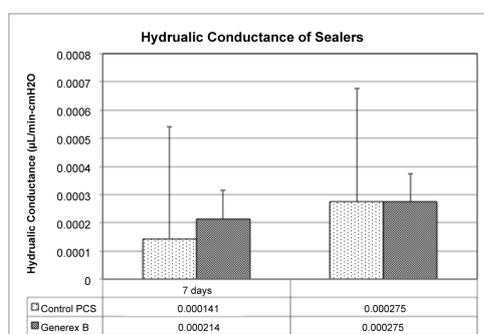

Figure 3b: Hydraulic conductance of materials tested for root canal sealers. Sealing was statistically unchanged with time but worsened for the ZOE based sealer significantly.

For the root canal sealer test, no significant differences could be identified between the Generex B (experimental) or Pulp Canal Sealer (control) at either 7 days (p=0.48) or 28 days (p=0.08). Nonparametric pair wise comparisons and application of the Mann-Whitney rank sum test to the control and experimental group results at 7 days revealed no significant differences (p=0.496). However, post-hoc multiple comparisons showed that for the factor "Time interval", the difference between the 7-day and the 28-day results for the control group was highly significant (p=0.005), with the 28-day leakage being worse than the 7-day leakage. Conversely, the difference between the 7-day and the 28-day results for the Generex B group was not significant (p=0.095). However, application of the same test for the 28 days results resulted in a significant difference between the two groups (p=0.045), with less leakage exhibited by the Generex B sealer. No leakage was observed in the negative control specimens. Root canals that were filled with gutta-percha leaked 38-59 times as much as when the root canals were filled with gutta-percha and either sealer. Unfilled root canals leaked 130,000-200,000 times as much as when the root canals were obturated with gutta-percha and sealer.

Discussion

The Generex A material was easy to place, stable, and resistant to rinsing, as had also been noted by Porter [16]. The Generex B material had the elastic behavior that is characteristic of other root canal sealers. The enhanced workability of the experimental Generex A and B materials may have been a result of the powder formulas, the perceptibly finer particle sizes, or the gels, as compared to MTA powder mixed with water. Generex A had a shorter working time, less flow, higher film thickness, and higher strength than the Generex B sealer. The MTA samples had a more crumbly texture for placement. The Generex A and MTA properties were suitable for its intended use as a vital pulp and root treatment material, which does not need low film thickness. These handling results are similar to Camilleri [22] who achieved better handling by adding a superplasticizing ingredient to the water for mixing with calcium silicate powders, and Ber [18] improved handling of MTA powder with calcium chloride salt and methyl cellulose. The conformance of a prototype calcium silicate-based sealer to the ISO 6876 or ADA 57 requirements have been published [22], but that sealer did not meet the requirements, such as film thickness. This experimental Generex B sealer did met the ADA 57 specification for all characteristics with the exception of solubility after 24 hours in the ADA 57 test method for sealers. However, the solubility requirement was met when the sealer was allowed to cure 120 hours. The Generex B sealer contains primarily calcium silicates, which are known to gradually strengthen over a month [13]; therefore, solubility testing after longer setting times for this lower powder to liquid ratio seemed appropriate.

Both Generex materials and MTA had long setting times, ranging from 1 to 24.5 hours, depending on the technique, but all of which would occur after any dental procedure is completed. A short setting time is not desired or required for root canal sealers, although shorter setting times are preferred for the vital pulp and root treatment procedures. In another study, Generex A material was reported to set in 1.25 hours in a non-plaster mold [16]. A setting time of 2.75 hr was reported for experimental gray MTA [13]. Others have measured 14 min setting time for MTA Angelus [36], but much longer for white ProRoot MTA with setting times up to 2.5 hr [37], which have similar compositions. All these aforementioned setting times are considerably less than measured for these experimental materials. The non-absorbent mold and the plastic sheets on the surface probably contributed to the long setting times seen here, even though this technique was chosen to avoid varying the water to cement ratio as the material was setting, by wicking the moisture of the mixed calcium silicates into the acclimatized plaster mold. Using a non-porous mold may not characterize the in vivo conditions. The test method was critical for measuring the setting for water-based cements. These calcium silicate-based materials set in longer than 1 hour. Nevertheless, the contrast was clear that the Generex materials were washout resistant before setting unlike MTA. This difference in handling made the sealer usable and the root end material more convenient. The working time of the Generex materials was much shorter than for ProRoot MTA, due to the washout resistance imparted by the new formulas. The shorter working time for the Generex materials could compensate clinically for their longer setting time.

The radiopacity values reported for other MTA materials have ranged from 3 to 8.5 mm thickness of aluminum [13,38,39]. In this work, white ProRoot MTA had an equivalent 5 mm and the new materials had slightly higher radiopacity at 7 and 6 mm of aluminum for Generex A and B. The radiopacity had been reported for Generex A was 6.8 mm for a non-uniformly blended sample [16], within the range reported here as 7 ± 0.5 mm. Although digital and film techniques would be assumed to give similar results when a standard

step wedge is included, some authors have noted discrepancies [40], which may contribute to the variations.

Generex A and white ProRoot MTA were previously reported [16] to have lower compressive strengths of 39 and 27 MPa, respectively, than reported here for Generex A at 69 MPa, with all measurements made after 7 days of setting. Small samples and high powder to liquid ratios require tight packing; defect-free samples are difficult to make. Higher strengths were measured in this study and Generex A material met the ISO 9917 requirement for 50 MPa compressive strength. The present measurements for Generex A were equal to the reported compressive strength after 7 days of setting for white ProRoot MTA [41], 67 MPa, and were not significantly lower than measured for gray ProRoot MTA, 82 ± 25 MPa [41]. Experimental gray MTA [13] had a strength of 67 MPa after 28 days, and white ProRoot MTA had a strength of 46 MPa after 3 days [37], which are reasonably comparable to the present Generex A strength, given that much of the strengthening occurs in the first 7 days of setting of calcium silicates. The new formulas for Generex A had roughly comparable strength to MTA materials. Generex B root canal sealer did not meet the ISO 9917 criteria for compressive strength, but compressive strength is not a requirement for sealers. However, a significantly higher bond strength to dentin has been published for Generex B, compared to other popular root canal sealers [26]. Although root-end filling materials do not bear any direct occlusal load [37], their compressive strength and bond strength to dentin can be important when used in large root perforations or resorptive defect repairs [42].

The literature is replete with measurements about the heavy metal contents for MTA-type products, [43] varying from less than 2 ppb [43] to 3.3 to 8.6 ppm [14], and 50 ppm for total arsenic [44]. Some studies report the leachable arsenic and lead tests of ISO 9917 and others reported the total arsenic and lead contents. This issue has arisen because the MTA patent described the use of industrial grade portland cement, and industrial materials can have relatively high contents of arsenic from using "waste" raw materials or low-grade fuels for manufacture [45]. The two experimental materials evaluated in this study conformed to the ISO standard as having no detectable lead (Pb) and less than 2 ppm of leachable arsenic.

Sealing

Within the limits of these leakage tests, the 2 sealers were equal in sealing after 7 days. After 28 days, the zinc-oxide-eugenol Kerr Pulp Canal sealer had statistically worse leakage than at 7 days, unlike Generex B. However, Generex B contains tricalcium silicate, a well-known component of hydraulic cement, which gradually hydrates over 28 days.

Significant differences were measured in fluid leakage among Generex A, White ProRoot MTA and SuperEBA, the latter 2 materials being commercially available, frequently advocated, root-end filling materials. The more extensive fluid leakage observed in the Super EBA group versus the White ProRoot MTA group confirmed previous dye leakage [46, 1, 47] and bacterial leakage results [48, 10, 49]. Both the White ProRoot MTA group and the Generex A exhibited minimal fluid flow through the root-end fillings. The fluid flow may represent the permeability of water through the matrices of the set materials. Further sealing may have occurred by the bioactivity (precipitation of apatite) from the tricalcium and dicalcium silicate component of Generex A and ProRoot MTA that occurs in the presence of phosphate-containing fluids [3, 50]. The hydraulic conductance values in both groups were minimal and not significantly different from one

another at seven days. However, a significant difference was observed, at least with the pairwise Mann-Whitney analysis of the leakage results between the Super EBA control material and the tricalcium silicate Generex A after water storage for 28 days. Furthermore, the results of the Wilcoxon ranked sum tests indicated that the control group exhibited considerable difference in the 7-day and 28-day hydraulic conductance results, while the respective 7-day results from the experimental group were not significantly different from the 28-day results. These differences were not evident in the two-way repeated measures ANOVA, probably due to the lack of a sufficient specimen number that is necessary for this more robust analysis.

The null hypothesis was rejected of no difference in the seven-day and the twenty-eight days leakage results between the control zinc oxide eugenol root canal sealer and the Generex B root canal sealers. The deterioration of sealing quality in the control sealer with time, confirmed by both the two-way repeated measures ANOVA and the pairwise Wilcoxon ranked sum test, may be attributed to the leaching of incompletely reacted free eugenol or the hydrolysis of the zinc eugenolate chelate to zinc hydroxide and free eugenol from the set sealer [51-53]. The solubility of MTA cements, on the contrary, is more controversial, ranging from negligible solubility [8], 1.76-2.83% [54], to a projected 22.1-31.1% [55] depending on the water/powder ratio and the period of water immersion. In this study, the experimental sealer, when used with gutta-percha, did not generate a 100% leak-free seal, as the set material is potentially porous [55]. These results are reinforced on the sealing quality of MTA in various applications [56-60]. Similar to the use of other root canal sealers, it appears that the creation of a secondary coronal seal would complement the clinical use of the experimental MTA sealer to prevent coronal leakage. However, the potential merit of the experimental MTA sealer lies in the fact that the apical seal it generates is equivalent to that of the control Pulp Canal Sealer at 7 days, and does not further deteriorate with water storage at 28 days.

Conclusions

Two new tricalcium silicate-based materials (Generex A and B) had higher flow, higher radiopacity and lower film thickness than other MTA-type products. The Generex A vital pulp and root treatment and the Generex B root canal sealer conformed to the ADA 57 and ISO 9917 requirements as they apply to the intended uses.

Generex A was statistically better than Super EBA, and equal to MTA in a sealing test. Generex B had had statistically significantly less leakage after 28 days of water storage compared to a common ZOE sealer. The sealing tests also added evidence for the suitability of these materials for testing in animal models.

Acknowledgements

potential conflict of interest is disclosed. **JLM:** I have a consultancy with Dentsply Tulsa Dental Specialties, from whom these materials were received. **FRT:** I have tested materials under contract with Dentsply Tulsa Dental Specialties, from whom these materials were received.

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