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ORIGINAL ARTICLE

In vitro evaluation of bond strength and sealing ability of a new low-shrinkage, methacrylate resin-based root canal sealer

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KEYWORDS
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Background/Purpose: The aim of this study was to evaluate a new low-shrinkage, methacrylate resin-based root canal sealer (LSRCS) to determine its bond strength in radicular dentin and sealing ability.
Methods: Extracted single-root teeth were randomly divided into three experimental groups ($n = 20$) for obturation with Gutta-percha (GP)/AH Plus, Resilon/Epiphany, or Resilon/LSRCS. One-half of each experimental group was analyzed by the push-out test, using sections perpendicular to the long axis divided into 1 mm serial slices and a universal testing machine to detect the loading force. The other half was analyzed by the dye penetration test using 2% methylene blue solution (pH = 7) and measuring dye leakage under a stereomicroscope.
Results: The push-out test revealed significant differences ($p < 0.05$) in bond strength produced by the three sealers; the GP/AH Plus group showed the highest bond strength, followed by Resilon/LSRCS and Resilon/Epiphany. According to the microleakage data, GP/AH Plus showed the least dye penetration, which was significantly less than Resilon/Epiphany and Resilon/LSRCS. There was no difference in apical leakage between Resilon/Epiphany and Resilon/LSRCS.
Conclusion: The newly developed LSRCS, although not superior to AH Plus in bond strength or sealing ability, possesses monoblock potential and application prospects.

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Introduction

Root canal sealer (RCS) is crucial to obtain perfect obturation of the intricate root canal anatomy and to compensate for core materials limited bonding abilities to the radicular dentin. The ideal RCS will completely attach the defective bonds, entomb the remaining bacteria, and achieve a hermetical seal of the root canal system.[1] The indispensible criteria of an ideal RCS include: effective adhesion to both the radicular dentin and the core root canal filling materials, no shrinkage upon setting, biocompatibility, and suitable working time.[2] Methacrylate-based RCS were clinically introduced in the mid-1970s and have since experienced four generations of development, evolving from non-etching to self-etching adhesion potential.[3] Although they have imperfect physiochemical properties and biocompatibility, the resin-based sealers have remained the popular choice for creating an impervious seal, due to their excellent bonding ability. More recently, the dual-curable methacrylate sealer Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA) was developed for use with Resilon core materials (Resilon Research LLC, Madison, CT, USA) to achieve a monoblock structure of a continuous resin bonded to the dentin wall, thereby providing an alternative to the conventional root canal filling system.[4]

After a root canal procedure, normal contraction stress along the interfaces of the radicular dentin seal can decrease the bond strength of the adhesive system. Polymerization shrinkage which occurs during setting, when monomer molecules are converted into a crosslink-network, is one of the most critical factors affecting resistance to contraction stress.[5] Bisphenol-A-diglycidyl dimethacrylate (Bis-GMA) is the most commonly used base monomer in methacrylate RCS. This bulky dimethacrylate monomer (molecular weight = 512) was synthesized by Bowen in 1965, and features relatively low polymerization shrinkage, rapid hardening, and low volatility[6]; however, the methacrylate sealer is subject to microleakage, which has been implicated in eventual clinical failure. To reduce the microleakage, several Bis-GMA analogues and substitutes have been developed which produce less shrinkage in the setting phase. For example, Khatri et al synthesized urethane derivatives of Bis-GMA which exhibited lower viscosity and higher hydrophobicity than Bis-GMA.[7] Ge et al later developed another low shrinkage methacrylate monomer by adding bulky substituent groups, to facilitate improved double bond conversion and decreased polymerization shrinkage.[8] Thus, it was demonstrated that increasing the molecular weight of the monomer represented an effective strategy to decrease polymerization shrinkage.

In more recent efforts to further diminish the polymerization shrinkage, He et al synthesized a novel, low-shrinkage and high molecular weight (574) dimethacrylate monomer 4′4-AMBHMB.[9,10] A system using 4′4-AMBHMB mixed with the dental resin monomer triethylene glycol dimethacrylate (TEGDMA) produced less polymerization shrinkage, and featured decreased solubility and water sorption as compared to the Bis-GMA/TEGDMA commonly used in resin-based RCS. In our most recent work, the 4′4-AMBHMB/TEGDMA (50:50 optimum proportion) was used to create a new low-shrinkage, methacrylate resin-based RCS (LSRCS). The composition of LSRCS also included the monomers (N,N-dimethylamino)ethyl-methacrylate (DMAEMA) and camphorquinone (CQ), at optimum proportion of 2:4, and the fillers hydroxyapatite (HA), barium sulfate (BaSO4) and zinc oxide (ZnO), with optimum proportion of 9:3:2. The LSRCS displayed good physicochemical properties and biocompatibility.[9–11] Therefore, we extended our study to evaluate the interfacial bond strength and apical sealing ability of this newly synthesized resin-based sealer, and perform comparative analysis between LSRCS and the commercial RCSs AH Plus and Epiphany.

Materials and methods

Selection of teeth

For this study, intact, caries-free human maxillary incisors with single-straight roots, which had been extracted for periodontal reasons, were selected. Informed consent for the use of the biosample in scientific research was obtained in conjunction with study approval by the Ethics Committee of Guanghua School of Stomatology, Sun-yat Sen University. Teeth were carefully examined for open apices, cracks, or resorptive defects, which led to sample exclusion. The included samples ($n = 75$) were stored in 0.9% sodium chloride containing 0.02% sodium azide at 4 °C until use. All the roots were radiographed to confirm root canal patency and single. Crowns were sectioned perpendicularly to the long axis at the cemento-enamel junction using a water-cooled fissure bur to make the length proximal to 14 mm.

Instrumentation and obturation of root canal

The root canals were accessed by using a size 15 K-file until visualized at the apical foramen. The working length of each root was established by subtracting 1 mm from the foramen. The canal spaces were mechanically shaped by using rotary ProTaper nickel-titanium files (250 rpm; Dentsply Maillefer, Tulsa, Oklahoma, USA), following the manufacturer's instructions and using a crown-down technique. The preparation of the apical third was completed by use of a ProTaper file F3. All canals were irrigated with 10 mL of 5.25% sodium hypochlorite (NaClO), followed by a wash with 10 mL of 17% ethylenediaminetetraacetic acid (EDTA). Finally, the root canals were flushed with 10 mL of distilled water to remove any residual EDTA or NaClO, and blot-dried with sterile paper points.

The 75 root samples were randomly divided into three experimental groups ($n = 20$; for obturation with Gutta-percha (GP)/AH Plus, Resilon/Epiphany, or Resilon/LSRCS) and three control groups ($n = 5$). Each experimental group was subdivided into two groups ($n = 10$; for push-out or dye penetration test). All the roots were filled using the lateral condensation technique. Group 1 roots were filled with AH Plus and 0.04 taper GP points. Group 2 roots were painted with self-etching primer for 30 seconds using microbrushes and paper points to remove the excess primer; then, Epiphany was applied to the canal, followed by 0.04 taper Resilon points. Group 3 roots were filled as in group 2, but

LSRCS was substituted for Epiphany. The components of the three sealers are shown in Table 1. For the control group, unfilled 0.04 taper GP points were used as control for the push-out test, while filling with GP only or GP/AH Plus was used as the positive and negative controls, respectively, for the dye leakage test. Obturation light-curing for 40 seconds with a light emitting diode (LED) was performed on all roots filled with Epiphany or LSRCS. The roots were then photographed to confirm the compact obturation and stored in a 100% humidified container at 37 °C for 7 days to ensure complete setting of the filling materials.

Preparation of root slices and push-out test

For the bond strength test, each tooth was embedded in epoxy resin and sectioned perpendicularly to the long axis in the coronal to apical direction, by using a slow-speed saw (Isomet; Buehler, Lake Bluff, IL, USA) under water cooling to obtain 1 mm serial slices. The thickness of 50 slices ($n = 15$ for experimental group, and $n = 5$ for control group) was confirmed by measuring with calipers. The circumferences of coronal (Cc) and apical (Ca) aspects of each slice were determined by an AutoCAD software program (AutoCAD 2004) using stereomicroscope digital images.

Each slice was subjected to compressive loading *via* a universal testing machine (Zwick/Roell 2010, Ulm, Germany) equipped with a 1 mm diameter cylindrical plunger, which contacted only the root filling. The loading force (N) was applied at a crosshead speed of 0.5 mm/minute in an apical to coronal direction, in order to eliminate any interference from normal root canal taper. Failure was indicated by extrusion of the intact cone of the root filling from the root slice and confirmed by the appearance of a sharp drop along the load/time curve. The area of the filling materials/dentin interface was approximated by the formula $[0.5 \times (Cc + Ca) \times h]$, where $h =$ the thickness of root slice. The bond strength (MPa) between the interfaces was calculated by dividing the failure value (N) by the interfacial area (mm^2).

Scanning electron microscopy

Representative samples of debonded or split surfaces from each group in the push-out test were processed by incubation for 3 minutes in 5.25% NaClO and 3 minutes in 17% EDTA, to remove the organic and inorganic debris from the dentin surface. The specimens were then dehydrated by soaking in an ethanol gradient series of 30%, 50%, 70%, 90% and 100% (twice in every concentration), and displaced by soaking in tertiary butyl alcohol and cryodessication for 4 hours. Finally, specimens were mounted on aluminum stubs, sputter coated with gold-palladium, and observed under a field emission-scanning electron microscope (FE-SEM JSM-6330F; Hitachi, Hsinchu City, Japan).

Dye penetration

To determine the apical dye leakage, all the experimental groups root surfaces (up to 2 mm distance from the foramen) were coated with two layers of fingernail vanish.

Table 1 Compositions of various root canal sealers used in this study.

Sealer	Material group	Chemical composition
AH Plus (Dentsply, USA)	Epoxide bond	epoxy resin calcium tungstate zirconium oxide aerosol iron oxide adamantine amine bisphenol-A-diglycidyl ether silicone oil
Epiphany (Sybronendo, USA)	Multi-methacrylates	bisphenol-A-glycidyl methacrylate ethoxylated Bis-GMA urethane dimethacrylate resin silane-treated barium borosilicate glasses barium sulfate silica calcium hydroxide bismuth oxychloride with amines peroxide, as a photoinitiator stabilizers pigment
LSRCS (new synthesized sealer)	Multi-methacrylates	4′4-AMBHMB triethylene glycol dimethacrylate (N,N-dimethylamino)ethyl-methacrylate camphorquinone hydroxyapatite barium sulfate zinc oxide

The negative control group root surfaces had the entire surfaces (including the 2 mm of tissue to the foramen) coated with fingernail vanish. The apical portion of the root, at 5 mm from the foramen, was immersed in 2% methylene blue solution (pH = 7.0) and incubated at 37 °C for 7 days. Afterwards, all the roots were thoroughly washed with running water and the varnish was removed by scraping with a scalpel. The clearing technique was carried out by an initial step of decalcification with 10% nitric acid; the solution was refreshed every 24 hours until the root was able to be punctured by a #25 syringe. Next, the specimens were rinsed in running water, dehydrated in an ethanol gradient series, and finally cleared and stored in methyl salicylate to make the roots transparent. To quantitate dye leakage, computer-assisted analysis (Image-Pro PLUS Version 5.1 software, Austria) was carried out on microscopic images (Stemi 2000-C, Zeiss, Germany). The longest leakage in the filling materials was determined from both the buccolingual and mesiodistal directions, and the mean was considered to represent the maximum leakage amount.

Statistical analysis

Data from the push-out and dye penetration tests, with normal distribution by the Kolmogorov-Smirnov test, were analyzed by one way analysis of variance (ANOVA) and the Tukey test to determine the statistical significance of intergroup differences ($p < 0.05$).

Results

Comparisons of adhesion strength

The mean value and standard deviation of the push-out test for each group is shown in Table 2. The data were normally distributed and ANOVA revealed a significant difference in mechanical adhesion strengths among the sealers ($p < 0.001$); the GP/AH Plus group showed the highest bond strength (2.4472 ± 0.8650 MPa), followed by the Resilon/LSRCS group (1.9431 ± 0.8178 MPa) and the Resilon/Epiphany group (0.7755 ± 0.3304 MPa). Fig. 1 shows the SEM cross-sections of failure interfaces, and Fig. 2 shows an instance of sealer penetrating into the dentinal tubules.

Comparisons of apical sealing ability

The mean dye leakage values of each group are shown in Table 3. ANOVA revealed a significant difference in apical sealing ability existed among the sealers ($p = 0.001$). The GP/AH Plus group exhibited the least amount of microleakage

Table 2 Push-out bond strength of sealers.

Group	Root canal filling materials	n	MPa, mean ± SD
1	Gutta-percha/AH Plus	15	2.4472 ± 0.8650
2	Resilon/Epiphany	15	0.7755 ± 0.3304
3	Resilon/LSRCS	15	1.9431 ± 0.8178
4	Gutta-percha	5	0.3920 ± 0.0696

AH Plus = AH Plus® is a brand name of root canal sealer; LSRCS = low-shrinkage resin root canal sealer.

(0.72 ± 0.19 mm), as compared to Resilon/LSRCS (4.46 ± 1.99 mm) and Resilon/Epiphany (4.52 ± 1.41 mm) groups. There was no statistical difference between the Resilon/Epiphany and Resilon/LSRCS groups ($p > 0.05$). The positive control group had microleakage along the whole length of the root canals, while the negative control group had no detectable apical microleakage. The microleakage of the GP/AH Plus group was not statistically different from that of the negative control group ($p > 0.05$).

Discussion

Epoxy resin RCS of the AH series is in wide clinical use with long-term dimensional stability, sufficient flow, good biocompatibility, and sealing ability.[12,13] However, the less than optimal adhesion to core materials (GP) limits the overall sealing performance of this type of sealer. The Resilon/Epiphany resin-based obturation system was developed to overcome this limitation by forming a "monoblock", and canals filled with this system were reported to have increased resistance to both fracture and apical microleakage.[14] However, other studies found that Epiphany had equivalent adhesion and sealing abilities to AH Plus.[15,16] The push-out test in this study demonstrated that the bond strengths of GP/AH Plus and Resilon/Epiphany were significantly different, agreeing with previous studies which showed that AH Plus had higher bond strength than the methacrylated-based RCSs Epiphany and EndoREZ.[17,18]

Some studies have demonstrated that Epiphany undergoes substantial polymerization shrinkage, up to 3.54%,[19] and has weak bond strength to both Resilon and dentin.[20] It is known that increasing the molecular weight of the sealer's base monomer can decrease the potential and extent of polymerization shrinkage. The base monomer of the new methacrylate-based RCS LSRCS is 4,4′-AMBHMB, which features a higher molecular weight and a larger molecular volume than Bis-GMA (MW = 572 vs. 512, respectively). Resin systems based on low molecular weight monomers typically exhibit higher shrinkage value than those based on higher molecular weight agents,[21] and the new monomer 4,4′-AMBHMB exhibited less polymerization shrinkage than Bis-GMA.[10] In fact, the polymerization shrinkage of the 4,4′-AMBHMB-based sealer LSRCS was determined to be 1.83% (data not shown), which is smaller than either of the Bis-GMA-based sealers Epiphany or Epiphany SE.[22] The enhanced bond strength of LSRCS over that of Epiphany, may be due to its significantly decreased potential of polymerization shrinkage. It is important to note here that the SEM morphological analysis of the push-out test showed that some Resilon and LSRCS still remained in the radicular dentin, indicating that the bond strength of the two circumferential interfaces (one between LSRCS and radicular dentin, the other between LSRCS and Resilon) was more robust than the mechanical loading force applied to each. This finding might reflect the formation of a monoblock system, in which the Resilon, LSRCS, and dentin formed a completely homogeneous unit.

According to our study, the bond strength of the methacrylate-based sealer was not better than that of the epoxy resin sealer. This finding could be attributed to

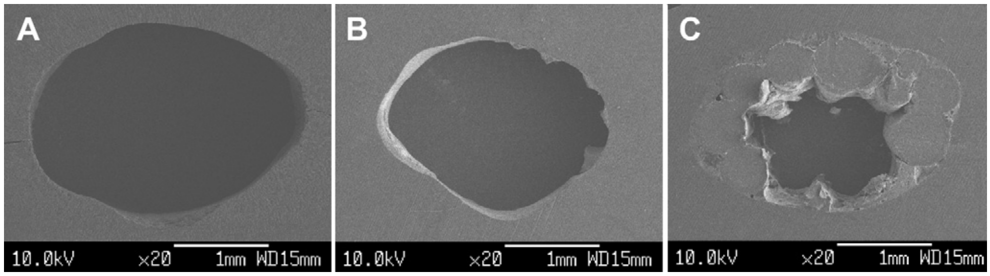

Figure 1 Push-out test results of Gutta-percha/AH Plus (A), Resilon/Epiphany (B), and (C) Resilon/LSRCS, as detected by FE-SEM. (A) The entire obturation was only slightly dislocated in the AH Plus group. A few remnants were present along the dentinal walls; (B) a filmy layer of Epiphany root canal sealer covered the dentin, without core materials, in the Resilon/Epiphany group; and (C) the core materials and sealer were tightly bonding to the root canal dentin, with no gaps, in the Resilon/LSRCS group.

the differences in polymerization which occurred during the curing process. As a self-cure epoxy resin sealer, AH Plus has a long polymerization process, which can facilitate stress relief by slow contraction.[23] Moreover, AH Plus then has sufficient time to extensively penetrate into micro-irregularities and dentinal tubules, and to react with any exposed amino groups in collagen, thereby increasing the mechanical interlocking between sealer and dentin.[19] The substantially faster curing time of Epiphany and LSRCS (40 seconds by LED light) may have generated incomplete infiltration into the demineralized dentin, which would otherwise form the hybrid layer. In addition, the oxygen molecules along the dentinal walls and tubules may inhibit free radicals polymerization and produce polymers with uncured monomers.[24]

Although any polymerizing endodontic sealer will be subjected to large polymerization stresses during setting, which may cause debonding and gap formation along the periphery of the root filling materials and result in low bond strength to radicular dentin, the newly developed LSRCS was considered to have high bond strength values, for the following reasons. Firstly, low polymerization shrinkage produced minor stress distribution, and reduced the degree of the breakdown among the carbon-carbon double bond, hydrogen bond and van der Waals forces in the sealer. Secondly, the total bond strength of dentin-bonding agents is the sum of strengths of the resin-tag, hybrid layer, and surface adhesion.[25] In our study, some resin-tags of LSRCS were observed by SEM to have penetrated into the radicular dentinal tubes (Fig. 2C), with no gaps in the LSRCS/dentin interface. Thus, around each resin-tag, a cylindrical hybrid layer had developed, which anchored the tag to the adjacent intertubular dentin.[25] For Epiphany, however, SEM showed that not only the outer portion of the resin tags was broken, but also the Epiphany/dentin interfaces (Fig. 2B). As a result, the new sealer LSRCS filled with Resilon was deemed to have a higher bond strength than the Resilon/Epiphany combination. Considering both the quantitative and morphological data, it appears that LSRCS adhesion properties are not inferior to those of AH Plus. Furthermore, LSRCS possesses monoblock potential, further increasing its application prospects.

Since leakage represents a major reason for failure in root canal therapy, the sealing ability of a RCS is considered a crucial feature; development and application of sealers with optimal sealing ability will ultimately improve the prognosis of root canal treatment. In our study, the dye penetration method demonstrated the leakage potential of the various sealers. AH Plus showed the least amount of microleakage in all experimental groups, and was similar to the negative control group. Epiphany and LSRCS appeared to be similar in their apical sealing abilities. While other investigators have also reported that the epoxy resin-based RCS provides better sealing properties than the methacrylate-based sealers,[15,26] just as many studies have reported that AH Plus and Epiphany have equal sealing properties.[27,28] Still, other studies have found that the apical sealing ability of Epiphany was superior to AH Plus, having observed absolutely no apical leakage with the Epiphany sealer.[29,30] Such contradictory results may be caused by the different root canal preparation methods used in each study, or the different canal filling techniques and microleakage testing models.

Figure 2 High magnification FE-SEM view showing penetration into dentinal tubules by the three resin-based root canal sealers. (A) AH Plus displayed long resin tags that had formed in the dentin tubules. The geometrical fillers of AH Plus were apparent; (B) Epiphany/dentin interfaces showed resin tags infiltrating into the dentinal tubules with visible breakage formation; and (C) the LSRCS/dentin interface showed resin tags with no gaps.

Table 3 Dye penetration values of sealers.

Group	Root canal filling materials	*n*	mm, mean ± SD
1	Gutta-percha/AH Plus	10	0.72 ± 0.19
2	Resilon/Epiphany	10	4.52 ± 1.41
3	Resilon/LSRCS	10	4.46 ± 1.99
4	Gutta-percha	5	14
5	Gutta-percha/AH Plus	5	0

AH Plus = AH Plus® is a brand name of root canal sealer; LSRCS = low-shrinkage resin root canal sealer.

Thorough irrigation of the canal system is essential to create an environment favorable to successful obturation, and ultimately to clinical success of the root canal procedure.[1] EDTA is a common clinically applied chelating agent which is capable, when used in conjunction with NaClO, of effectively removing the smear layer. Since the self-etching sealers are incapable of creating a hybrid layer,[31] the NaClO-EDTA irrigation protocol was used in this study. LSRCS was able to penetrate into the radicular dentin tubules, as evidenced by long resin tags (data not shown), indicating its ability to permeabilize demineralized dentin. Thus, the LSRCS features of low polymerization shrinkage, high bond strength to radicular dentin and favorable infiltration, are expected to facilitate superior apical sealing ability, as compared to Epiphany. However, the dye penetration results clearly demonstrated that LSRCS and Epiphany produced similar microleakage. This finding may be due to the core material Resilon, which is susceptible to enzymatic hydrolysis[32] and biodegradation by bacterial/salivary enzymes.[33] It is likely that endodontically-relevant bacteria play a role in the apical or coronal leakage event, possibly compromising the seal achieved after root canal treatment. Furthermore, the amount of dimethacrylate (polycaprolactone/dimethacrylate is 10:1)[20] in Resilon is considered insufficient to form strong bonding to LSRCS, creating microleakage potential between the resin and filler.

In conclusion, the newly synthesized LSRCS was superior to Epiphany in bond strength to radicular dentin and the core material Resilon, and had an apical sealing ability that was as effective as that of Epiphany. Although LSRCS did not exceed the AH Plus bond strength or sealing ability, it was shown to have monoblock potential, unlike AH Plus; thus, LSRCS possesses potential for beneficial clinical applications. Future development of improved core materials which complement LSRCS will likely enhance the overall success of the root canal procedure.

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