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# Assessment of marginal integrity of five different polymers in retrograde root-end filling following apicoectomy: An *in-vitro* analysis with scanning electron microscope and micro-radiography

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*Purpose*: The aim of the study was to evaluate marginal adaptation properties of five different sealers used as root filling material. *Material*s: Apical resection was performed on 100 canals of 50 maxillary premolars in slaughtered pigs. The root end of 24 canals were retrogradely filled with Diaket™, 37 root canals with Super-EBA™ including 18 with Super-EBA™. Regular and 19 with Super-EBA™ fast, 20 canals with ProRoot® MTA and 19 canals with AH-Plus™. For quantitative examination, scanning electron microscope (SEM) analysis and for qualitative examination, microradiography was performed. *Results*: Diaket™ achieved an overall average of 4.872 microns gap value as the best result. The mean values of marginal gap widths of AH-Plus™ and Super-EBA™ were almost with 8.044 microns and 9.951 microns in about the same magnitude, followed by Super-EBA™ Regular with 11,560 microns. Highest marginal gap value was found for ProRoot® MTA with 18.343 microns ($p < 0.001$). *Conclusion*s: Both in terms of its marginal integrity and material properties, Diaket™ is the most suitable material for retrograde obturation. A preliminary version of the current paper could be found at www.researchsquare.com/article/rs-6198/v1.

*Key words*: *apical, endodontics, polymer, sealing, tooth*

## 1. Introduction

Recurrent periapical infections pose a substantial challenge to the dental practitioner. However, the use of novel methods and materials in periapical surgery has increased the success rates of treatment [44]. When routine endodontic treatment via coronal access cavity fails or is not feasible, and/or persistent contamination of the apical region occurs, apicoectomy combined with retrograde root-end filling is indicated [10], [29]. The aim of this procedure is to remove the apical root end with its lateral accessory root canals, remove the adjacent granulation tissues and obtain a well-sealed apical region with a biocompatible root-end filling material [16].

An ideal retrograde filling material should seal the apical region of the root canal to prevent bacterial leakage from the canal to the periapical tissues [4], [21], [26], [28]. The materials used for root-end fillings are: endodontic point systems (Silver, titanium-, ceramic- or acryl, which should be combined with a sealer), sealers (Zinc phosphate-, zinc eugenol-, glass ionomer cement, polyketon-based cements and mineral trioxid

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aggregate based cements, amalgam, gutta-percha (plastic substance from the latex of several Malaysian palaquium gutta) and gold.

Endodontic sealers for root-end fillings span many compositions and attributes with different properties. Organic materials of polyketone base show a high adhesive strength to dentin, are dimensionally stable and radiopaque. Before hardening of the material, they solve both fats and organic substances and absorb moisture remaining on the canal wall during setting [24]. They are characterized by a high wall adaptation, act also as a disinfectant [2], [36] and are one of the mostly evaluated material in the dental literature.

Epoxy-amine polymers are also widely used as endodontic sealer, due to their minimal shrinkage during curing and show outstanding, long-term dimensional stability [9], [18]. There is also no free formaldehyde leakage into the adjacent anatomical structures [19].

Thanks to their long-term satisfactory clinical results, zinc oxide eugenol-based root filling materials are also commonly used in the dental practice [6]. They have a dual system consisting of a powder and a liquid material and harden form constant, are radiopaque, have hydrophilic properties and are pH neutral [1], [6]. They basically have two different types, according to the curing time. The "Fast Set" can be used only with easily accessible operational fields due to the shorter curing time. Retrograde root tips are difficult to access, so that this material can be used only on easily accessible root tips. The "Regular Set" allows for a longer application due to its slower curing and, therefore, is suitable for application in the posterior region of the jaws.

Mineral trioxide aggregate (MTA)-based materials are popular products, which are form constant and radiopaque [11], [18]. The enclosed package contains distilled water and a powder, which forms a colloidal gel after mixing. According to Apaydin et al. [3], the materials could stimulate hard tissue formation in the adjacent periapical structures. Similarly to polyketone-based materials, the properties of MTA were tested in numerous studies and are suggested to be one of the most appropriate material for root-and root-end fillings [17], [35], [37]. Owing to their high biocompatibility, MTA resins are proclaimed to be suitable for retrograde root sealing following apicoectomy or intentional replantation [14], [15].

It is well known that the success of the endodontic treatment depends on the presence of a well-sealed apical region to prevent the spread of bacteria from the root canal to the surrounding tissues. However, it is not possible to define any of the above-mentioned materials as "ideal" yet. The aim of the study was to evaluate the marginal adaptation properties of five different sealers Diaket™, AH-Plus™, Super-EBA™ regular, Super-EBA™ fast und ProRoot® MTA used as root filling material following apicoectomy via scanning electron microscope (SEM) and microradiography *in vitro*.

# 2. Materials and methods

## 2.1. Experimental subjects

For the experiments, following the ethical approval, 25 pine halves of male and female pigs were obtained from the slaughterhouse in Kiel-Wellsee-Germany. At the time of slaughter, animals were between seven and nine months old. The heads were stored frozen after decapitation at –21 °C and thawed in the refrigerator at 4 °C for 48 hours before performing the root resection and root canal filling.

## 2.2. Root-end filling materials

In this study, five root filling materials have been studied: Diaket™, AH-Plus™, Super-EBA™ regular, Super-EBA™ fast und ProRoot® MTA. The root filling materials are listed in Table 1, according to the product names, manufacturer and composition of the material.

## 2.3. Apicoectomy and root-end filling procedure

Apical resection was performed on 100 canals of 50 maxillary premolars. 48 hours before the operation, frozen pork jaw halves were thawed in the refrigerator at 4 °C. They were disinfected with Betaisodona® solution (Mundipharma GmbH, D-65549 Limburg).

An incision was made on the line between the mobile and the stationary mucosa with a no. 11 Scalpel and a full thickness mucoperiosteal flap was raised to expose the bone adjacent to the root tips. The bone was removed with a ball mill (0.5 mm) under irrigation with isotonic saline (0.9% NaCl) until the root tips were exposed.

The root tips were separated 2–3 mm away from the apex with a Lindemann cutter and in vestibulo-oral direction with an angle of less than 45°. Then

Table 1. The root filling materials according to the product names, manufacturer and composition of the material

| Product | Manufacturer | Composition | |
|---|---|---|---|
| | | Component A | Component B |
| Diaket™ | 3M ESPE AG<br>Dental Products<br>Seefeld, Germany | 97 % Zincoxid<br>3 % Bismuthphosphate | 76 % Propionylacetophenon<br>23,3 % Vinyl-Copolymer<br>0,5 % Dichlorophen<br>0,2 % Triethanolamin |
| AH-Plus™ | DENTSPLY DETRAY GMBH,<br>Konstanz, Germany | Epoxybisphenol<br>Calciumwolframat<br>Zirconoxid | Amino-Adamantan<br>N,N-Dibenzyl-5-oxanonan<br>Calciumwolframat<br>Zirconoxid |
| Super-EBA™ regular | BOSWORTH COMPANY, IL, U.S.A. | Zincoxid<br>Magnesiumoxid<br>Feldspar<br>Fluorspar | Aqua dest.<br>Zinc<br>Orthophosphoric acid<br>Aluminium |
| Super-EBA™ fast | BOSWORTH COMPANY, IL, U.S.A. | Zincoxid<br>Magnesiumoxid<br>Feldspar<br>Fluorspar | Aqua dest.<br>Zinc<br>Orthophosphoric acid<br>Aluminium |
| ProRoot® MTA | DENTSPLY TULSA DENTAL<br>U.S.A. | Tricalciumsilkat<br>Tricalciumaluminat<br>Calciumoxid<br>Silica | Aqua dest. |

Table 2. Distribution of the root-end fillings according to the material used. The inequality of the sample size results from the damaged samples during grinding process for micro-radiographically evaluation

| Material | Number | | | |
|---|---|---|---|---|
| | SEM (longitudinal section) | SEM (transversal section) | Micro-radiography | Total |
| Diaket™ | 5 | 5 | 14 | 24 |
| AH-Plus™ | 5 | 5 | 9 | 19 |
| Super-EBA™ regular | 5 | 5 | 9 | 19 |
| Super-EBA™ fast | 5 | 5 | 8 | 18 |
| ProRoot®-MTA | 5 | 5 | 10 | 20 |

retrograde cavities were prepared via dental hand piece (D-88400 Biberach/Riss INTRAmatic, KaVo Dental GmbH,) and washed with 3% $H_2O_2$ and 70% alcohol and then dried.

The root end of 24 canals were retrograde filled with Diaket™, 37 root canals with Super-EBA™ including 18 with Super-EBA™ Regular and 19 with Super-EBA™ fast, 20 canals with ProRoot® MTA and 19 canals with AH-Plus™ (Table 2). All materials were prepared and applied according to the manufacturers' guidelines.

## 2.4. Preparation of the samples for SEM

After completion of the root-end fillings, teeth were osteotomised with hammer and chisel en-bloque from the bone segment (Fig. 1) and stored in 4% formalin. The teeth were cut in half in vestibulo-oral direction and were placed in in phosphate buffered solution (pH 7.4 to Sörensen $KH_2PO_4/Na_2HPO_4$) (Walter GmbH & Co. KG, D-24116 Kiel) for 20 minutes. The procedure was repeated three-times.

The samples were trimmed with a wet grinder (Jean Wirtz, D-Düsseldorf Germany) and Silicon-carbide paper with a grain of 400 in the transverse or longitudinal axis at a predetermined cutting plane, before the surface could be polished to a high gloss with a grain of 1200–4000.

Before taking an impression of the roots with Silagum® AV light, an addition cross-linked silicone (DMG Chemical Pharmaceutical Factory GmbH, D-22547 Hamburg Germany), with 30% isopropyl alcohol was used to clean the surfaces. The impressions were poured with epoxy resin (Stycast (TE-adhesive technology, D-30165 Hannover Germany) and left at room temperature for 24 hours.

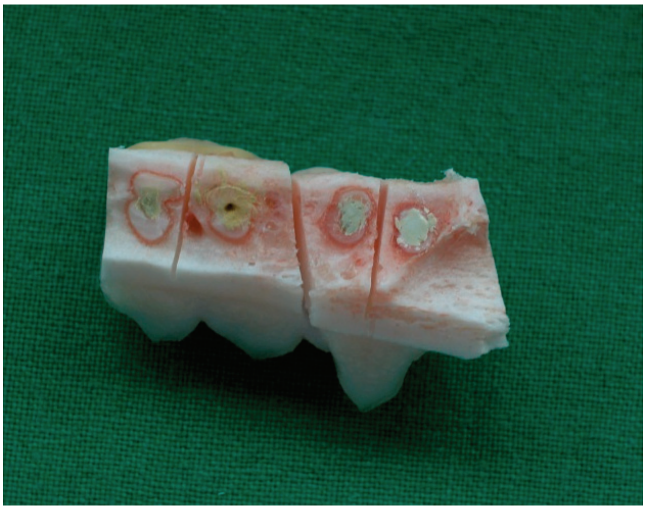


Fig. 1. After completion of the root-end fillings, teeth were osteotomised with hammer and chisel en bloque from the bone segment

## 2.5. SEM analysis

The replica models were placed on the aluminum specimen stubs (Agar Scientific, Ltd., Essex CM24 8 DA, UK) of the SEM. Electronic conductivity has been reached by vapor deposition of the samples with a 20 micron thin gold alloy at a pressure of 0.1 bar and at a voltage of 15 mA for 90 seconds under a vacuum.

To evaluate the samples obtained from the apical portion of the root tips, 60-, 190-, 320- and 600-fold magnification options were required on the screen of the SEM. To determine the value of the marginal gap, two points were determined in the area of greatest expansion and the distance was measured in microns.

## 2.6. Preparation of the samples for microradiography

After completion of the root-end fillings, teeth were osteotomized with hammer and chisel en bloque from the bone segment and stored in 4% formalin. With a band saw (Metabo, D-78822 Nürtingen Germany), the teeth were initially halved in vestibulo-oral direction, so that two root canals could be obtained individually.

The preparations were then placed over a period of two days in an embedder (PSI, CH-5232 Villingen) containing an alcohol, series (20, 40, 60, 80, 90%, 2 times 100% for each 45–60 minutes and finally 100% dehydrated for 6–8 hours) and then soaked in methacrylate. The samples were placed in a methacrylate solution another two weeks before they were placed in glasses capped with methacrylate and polymerized with fresh methacrylate solution in water bath at 38 °C. The polymerized samples were first cut with the band saw and the surfaces of the blocks were polished to 4000 with a Silicon-carbit paper with a grain size of 400, before the cutting plane for the actual recovery preparations was determined. Each sample was cut along its sectional plane with the band saw again, surface was polished, glued to a microscope slide and reduced with a precision saw (Leica GmbH, D-64625 Bensheim) to 100–200 micron thickness.

## 2.7. Microradiography

Via a wet grinding machine (Struers A/S, DK-2610 Rodovre) the samples were refined up to 70–110 micron and polished with a Silicon-carbit paper with a grain size of 800 to 4000. The samples were taken from the slide and put-on high-resolution microradiography plates in which the resolution is 2000 lines per millimeter (High Resolution plates (Kodak®, Rochester, NY 14650, USA). Subsequently, the exposure was performed in a Mikroradiographiekammer Faxitron 53855A (Hewlett-Packard, McMinnville, OR 97128, USA) at a focus distance of 16 cm. The voltage was set to 25 kV at a power of 3 mAs, exposure time of 6 minutes and 30 seconds for 70-micron samples and up to 10 minutes and 30 seconds for 110 microns film thickness. The exposure time was increased by increments of one minute per 10 microns thickness of the specimen.

The plates were embedded for five minutes in Kodak® HRP developer at 20 °C under constant movement swung (HRP developers /distilled water: 1/3). In a 1% acetic acid bath, the development was stopped after one minute. For fixation, Kodak® fixer 300A was used for 10 minutes with agitation (Kodak® fixer 3000A/distilled water: 1/3). This was followed for 15 minutes by washing with water and a final rinse with Agepon® (400 mL of distilled water and 2 ml Agepon®) for one minute. The plates were air dried and then covered with cover glasses (4 × 4.5 cm) using 1–2 drops of *n*-butyl acetate (xylene substitute) and a drop of Eukitt-air for 24 hours. After a drying period of seven days under an air extractor, the preparations were digitally photographed under a light microscope at a magnification of 1:18. The evaluation was performed using an image editing program (Adobe Photoshop 7.0 for Windows).

## 2.8. Statistical analysis

The statistical analysis was performed by using SPSS for Windows, version 14.0 (SPSS, Inc., USA). The continuous variables were analyzed using the Kolmogorov–

Smirnov test with respect to their normal distribution. In analysis of the abnormal distribution of the tested variables (Kolmogorov–Smirnov test: $p < 0.05$), non-parametric tests were used. Since the comparisons template more than two independent, not normally distributed random samples, the *H*-test were used by Kruskal and Wallis. A *p*-value < 0.05 was taken as statistically significant for all statistical tests. In the graphs, which were also created using SPSS; error bars were used to illustrate the mean values.

# 3. Results

## 3.1. SEM

For SEM analysis, 25 maxillary premolars were examined. Overall, root end filling was carried out on 50 canals following apicoectomies: 10 canals with Diaket™, 10 ™ with AH Plus, 20 with Super-EBA™ – including 10 with Super-EBA™ Regular and 10 with Super-EBA™ – and 10 with ProRoot® MTA. Axial and transversal cross sectional examinations were performed both on 25 canals.

### 3.1.1. Diaket™

*Longitudinal cross-sections*

In Figure 2a, marginal gap was measured at four points. The values were 1.440 microns and 2.160 microns. Adjacent to the Diaket™ – root end filling material, small bubbles were observed. These artifacts could be formed during the preparation of the material. In Figure 2b, the lower part of the apical filling margin is to be seen in 320× magnification. No marginal gap was recognizable.

In Figure 3a, in the sample with retrograde Diaket™, small bubbles could be seen. These artifacts could be attributed to the production of the replica models. The marginal gap was measured at four points. The measured marginal gap sizes were between 3,820 and 11,500 microns. In Figure 3b, 320 times magnification of the right side of the filling margin is shown. The material adheres very

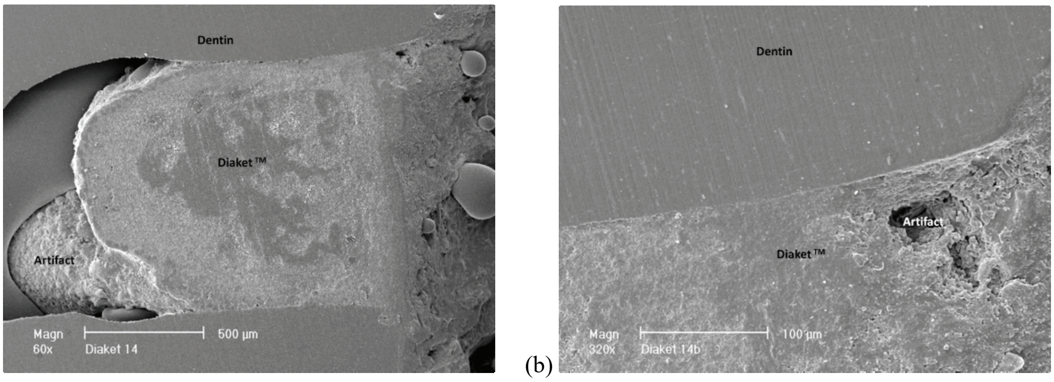
(a) (b)



Fig. 2. (a) 60× and (b) 320× magnifications on longitudinal plan revealed that Diaket™ obturated the root canal completely

*Transversal cross sections*

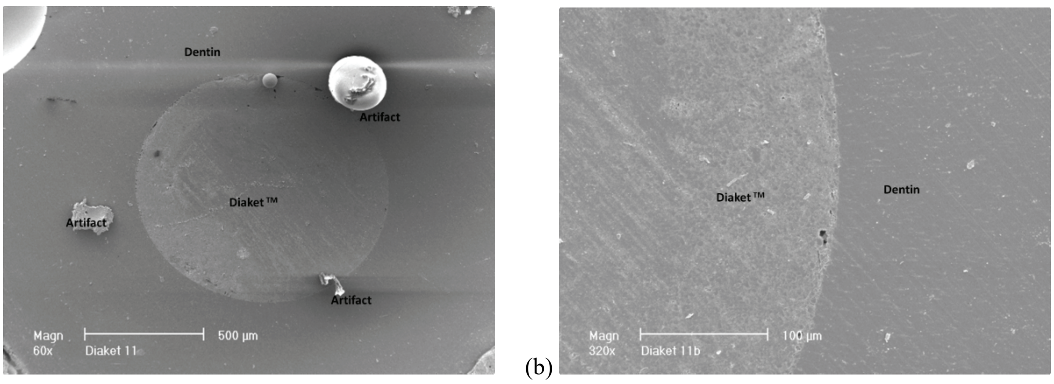
(a) (b)



Fig. 3. (a) Diaket™ on the transversal cross sections at 60× magnification on the transversal plane. The root canal is completely filled with the homogeneous mass, (b) at 320× magnification. Diaket™ adheres to dentin and shows a complete obturation

well to dentin and shows a complete connection without a marginal gap.

### 3.1.2. AH Plus™

*Longitudinal cross-sections*

In Figures 4a and 4b showing root canals with of AH Plus™ retrograde fillings in 60- and 320-times magnifications. The artifacts are thought to be formed during preparation of the replica models. The marginal gap sizes varied between 1.210 microns and 7.240 microns.

*Transversal cross-sections*

The four marginal gap sizes measured varied between 1,810 and 3,620 microns. In Figures 5a, b the filling margins with a very good marginal seal in 60- and 320-times magnifications are shown.

### 3.1.3. Super-EBA™ regular

*Longitudinal cross-sections*

In Figures 6a, 6b root canals with Super-EBA™ regular retrograde fillings at 60- and 320-time magnifications are shown. In Figure 6a, only small artefacts (bubbles) could be observed, which could have occurred by the preparation of replica models. Moreover, in Figs. 6a, b, longitudinal cut facets and air bubbles in the material can be clearly seen. The measured values ranged between 0.608 microns and 0.540 microns.

*Transversal cross-sections*

In Figure 7a, only small air bubbles can be observed, which were artifacts formed during the preparation of replica models. The measured gap values were between 1.000 microns and 2,560 microns. In Figure 7b, an enlargement of the upper filling margin could be observed, however, no gap can be detected on the edge.

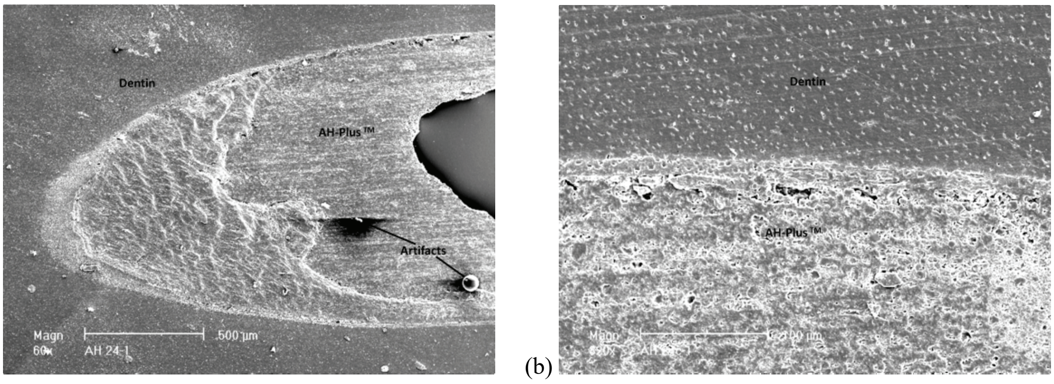
(a) (b)



Fig. 4. Retrograde filling with AH Plus™ in (a) 60× and (b) 320× magnifications on longitudinal plane.
The artifacts are thought to be formed during preparation of the replica models.
The marginal gap sizes varied between 1.210 microns and 7.240 microns

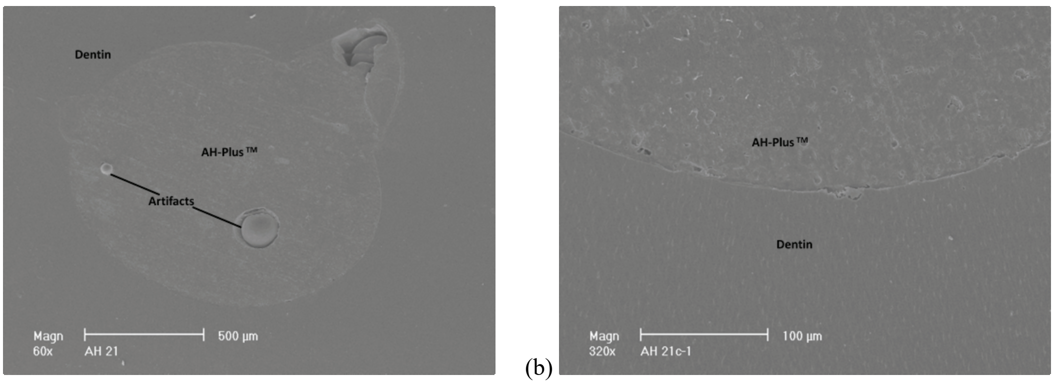
(a) (b)



Fig. 5. (a) AH-Plus™ viewed at 60× magnification on the transversal plane.
The root canal is almost completely obturated. The artifacts emerged during processing and not due to the material;
(b)AH-Plus™ at 320× magnification. The barely discernible edge gap proves an excellent adhesion
of the material to dentin despite its inhomogeneity

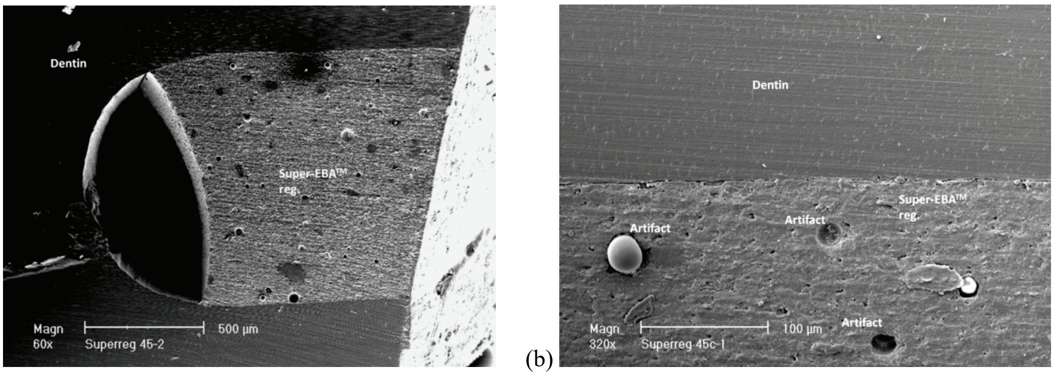
(a) (b)



Fig. 6. (a) Super-EBA™ Regular at 60× magnification on longitudinal plane.
The retrograde cavity was not completely filled by the material. The air entrapment can be clearly seen;
(b) Super-EBA™ regular at 320× magnification. A continuous minimal marginal gap could be seen

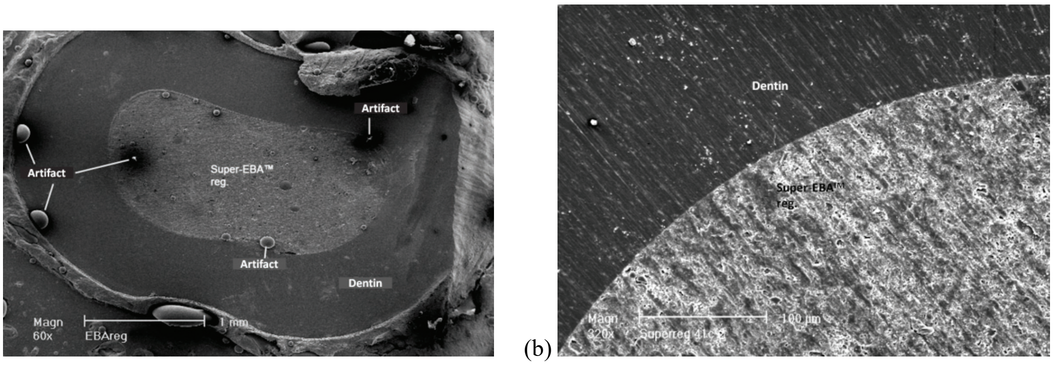
(a) (b)



Fig. 7. (a) Super-EBA™ Regular , at 60× magnification on transversal plane. The entire root canal was filled with material;
(b) Super-EBA™ Regular, at 320× magnification on transversal sections. No marginal can could be observed.
The difference between filling material and dentin could have emerged secondary to the coarse surface structure

### 3.1.4. Super-EBA™ fast

*Longitudinal cross-sections*

In Figure 8a, a small bubble in the middle of the specimen and small air pockets in the root end filling material (artifacts) can be seen. In Figure 8b, the left side of filling margin at 320-times magnification is shown. The measured gap sizes varied between 1.630 and 2.720 microns.

*Transversal cross-sections*

In Figure 9a, a bubble in the middle of the specimen can be seen. Super-EBA™ has almost bordered the edges without a gap on all sides of the dentine. In Fig-

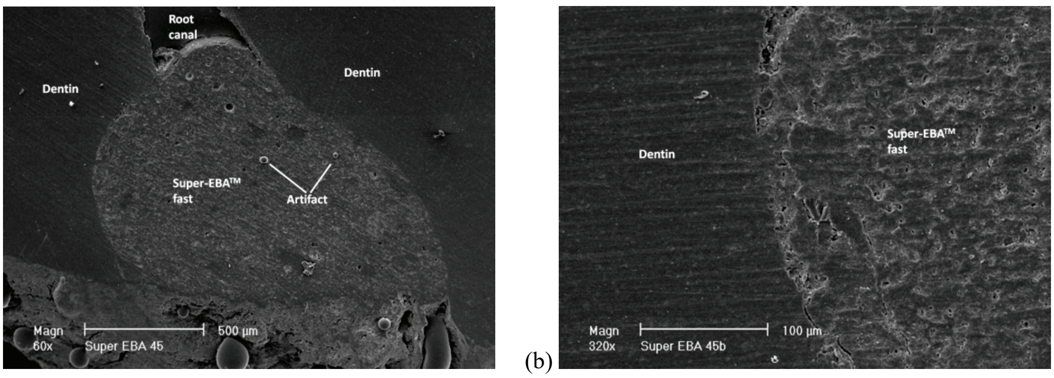
(a) (b)



Fig. 8. (a) Super-EBA™ fast at 60× magnification on longitudinal plane. The retrograde cavity was completely filled
by the material. Even at this magnification, a marginal gap could be recognized on the left side;
(b) Super-EBA™ fast at 320× magnification. The marginal gap which has been observed in Fig. 7a results from the lack
of adherence to the dentin wall and the inhomogeneity of the material could be recognized

ure 9b, a 600-time magnification of the right filling margin with longitudinal cut facets which have emerged during the polishing process of the tooth surface is shown. A marginal gap of 3.410 micron has been detected.

### 3.1.5. ProRoot® MTA

*Longitudinal cross-sections*

In Figures 10a, b, a sample in which the root end was filled with ProRoot® MTA at 60- and 320-times magnifications in the longitudinal section is shown. The root canal is located in the center. An artifact can be detected at the root tip. In Figure 8b, the top of the filling edge was enlarged. The gaps ranged between 13.300 and 23.000 microns.

*Transversal cross-sections*

In the center, the filled root canal and on its surface, small artefacts (bubbles) could be seen. (Fig. 11a).

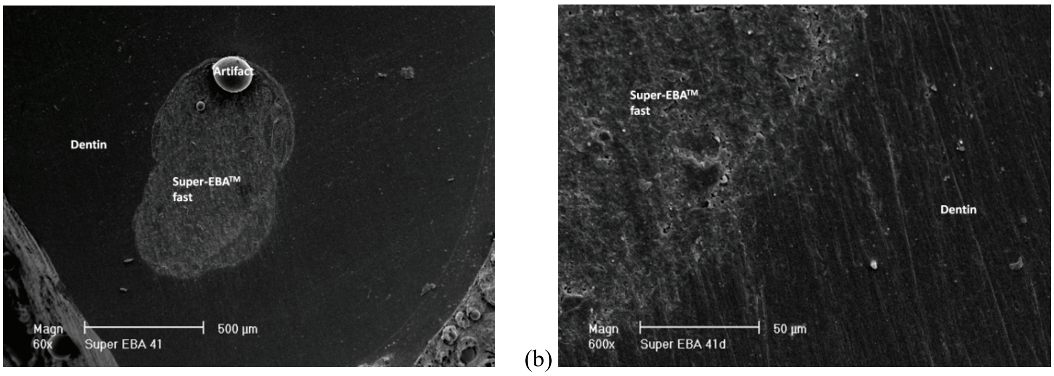



Fig. 9. (a) Super-EBA™ fast at 60× magnification on transversal plane. The root canal was completely sealed with the material; (b) Super-EBA™ fast at 600× magnification. An optimal connection between filler and dentin was observed. The facets were only at this high magnification recognizable

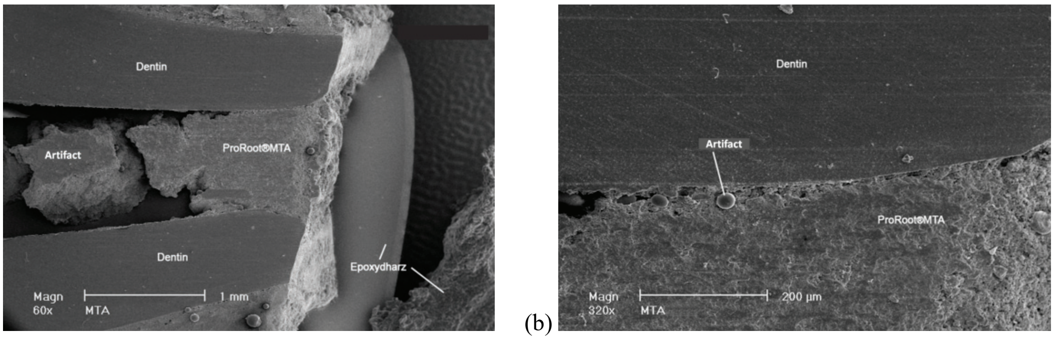



Fig 10. (a) ProRoot® MTA at 60× magnification on longitudinal plane. Marginal gaps and artifacts (secondary to the preparation) have been detected; (b) ProRoot® MTA at 320× magnification. The already recognizable marginal gap in Fig. 8a can be clearly seen

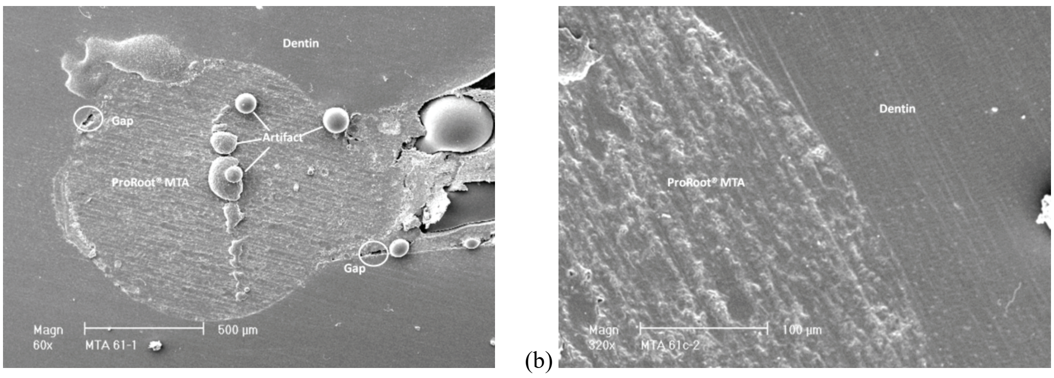



Fig. 11. (a) ProRoot® MTA at 60× magnification on transversal plane. Already at this magnification, marginal gaps along with numerous artifacts are clearly visible; (b) ProRoot® MTA at 320× magnification. An appropriate connection of the coarse-structured material with the dentin can be observed

In Figure 11b, the right fill margin was magnificated. The measured gaps ranged between 2.180 and 16,900 microns.

Comparative analysis of five materials revealed that, Diaket™ showed a mean gap value of 4.872 ± 4.09 microns and showed superior properties. The mean values of marginal gap values of AH-Plus™ and Super-EBA™ were almost with 8.044 ± 11.37 microns and 9.951 ± 14.36 microns in about the same magnitude, followed by Super-EBA™ Regular with 11,560 ± 16.60 microns. The highest marginal gap value has been detected by ProRoot® MTA with 18.343 ± 16.71 microns ($p < 0.001$) (Fig. 12).

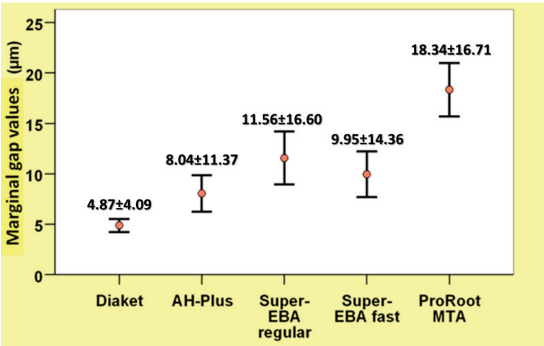



Fig. 12. Comparison of the marginal gap values of five different root-end filling materials. Diaket™ achieved an overall average of 4.872 microns gap value the best results and thus cut compared to the other materials significantly better

According to the results of SEM examination, the marginal gap values varied between 4.872 and 18.343 microns. In Table 3, the mean values of marginal gaps are illustrated.

Table 3. Mean marginal gap values of different materials

| Material | Mean | Standard deviation | Standard error | Median | *n* |
|---|---|---|---|---|---|
| Diaket™ | *a* | 4,096 | 0,648 | 3,600 | 40 |
| AH-Plus™ | 8,044 | 11,372 | 1,798 | 4,350 | 40 |
| Super-EBA™ regular | 11,560 | 16,603 | 2,625 | 4,325 | 40 |
| Super-EBA™ fast | 9,951 | 14,365 | 2,271 | 3,070 | 40 |
| ProRoot® MTA | 18,343 | 16,715 | 2,643 | 14,000 | 40 |
| Total | 10,554 | 14,074 | 0,995 | 4,610 | 200 |

## 3.2. Microradiography

For the microradiographic examinations, 25 premolars were used. Apicectomies were performed on 50 canals with subsequent retrograde root end fillings: 14 canals with Diaket™, 9 AH-Plus, 17 with Super-EBA™ – 9 with Super-EBA™ Regular and 8 with Super-EBA™ fast, and 10 with ProRoot® MTA.

### 3.2.1. Diaket™

In Figure 13, microradiographic analysis at 18× magnification of a retrograde filling with Diaket™ is shown. The material adheres to the dentin and no marginal gap could be detected between the sealer and dentin on the resected root apex.

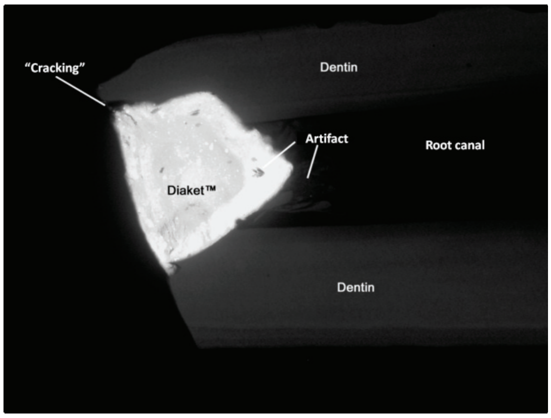



Fig. 13. Microradiographic evaluation of the root canal filling with Diaket ™ at 18× magnification. A "cracking" has formed in the filler during the preparation of the sample, which has not affected the marginal integration

### 3.2.2. AH-Plus™

AH-Plus™ appears as a non-homogeneous white-and-grey material in the center. The root end is not completely filled. The material-related air pockets formed during the filling of the root canal and has no influence on the obturation (Fig. 14).

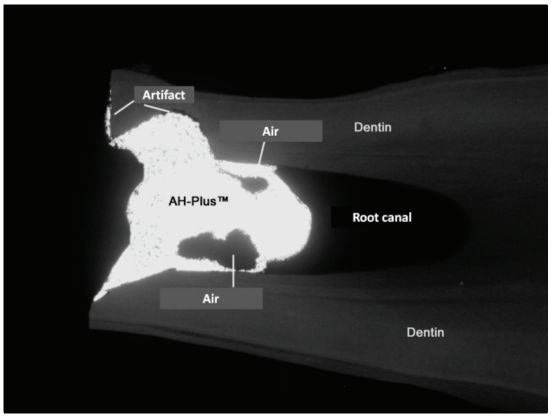



Fig. 14. Microradiographic evaluation of the root canal filling with AH-Plus™ at 18× magnification. The root canal could not be completely filled; thus, two large air pockets have emerged during processing

### 3.2.3. Super-EBA™ regular

Similarly to AH-Plus™, production-related artifacts within the sealer could be recognized. The black, elongated structure in the right half is a part of the not completely filled root canal. The marginal integrity was not complete (Fig. 15).

### 3.2.4. Super-EBA™ fast

The root filling material could be seen in the center as an inhomogeneous surface with dark inclusions. The marginal integrity between the dentin and Super-EBA™ was almost optimal (Fig. 16).

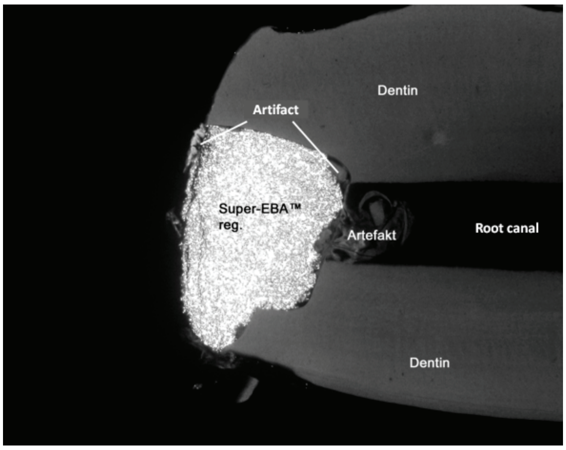



Fig. 15. Microradiographic evaluation of the root canal filling with Super-EBA™ Regular at 18× magnification. In addition to the artefacts detected centrally, the marginal gaps were clearly visible

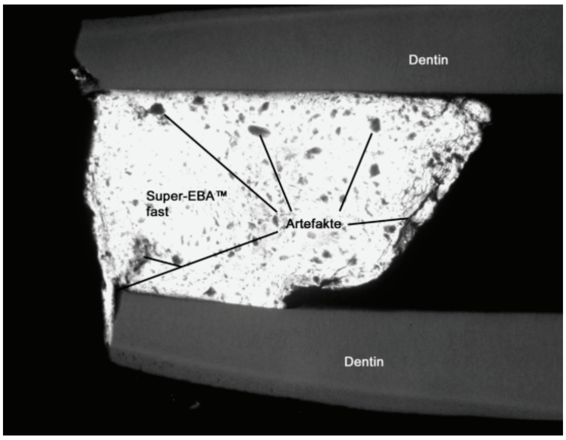



Fig. 16. Microradiographic evaluation of the root canal filling with Super-EBA™ fast at 18× magnification. The coarsely porous material adheres well to the dentin. The filling material is very rich in contrast

### 3.2.5. ProRoot® MTA

ProRoot® MTA showed a non-homogeneous structure. The marginal integrity was superior to all other materials evaluated (Fig. 17).

The results of microradiographic analysis confirmed for all materials a good marginal integrity and clean transitions of materials to the dentine at resected root apex.

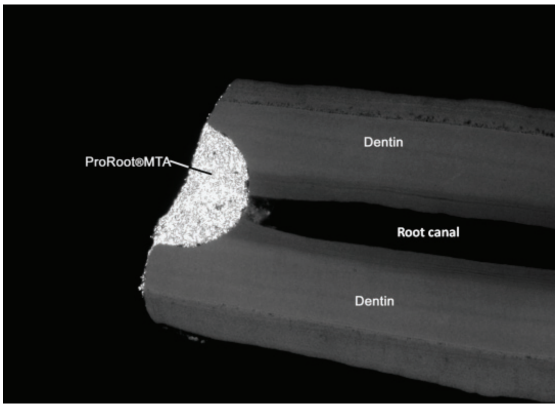



Fig. 17. Microradiographic evaluation of the root canal filling with ProRoot® MTA at 18× magnification. The retrograde preparation was completely sealed by the material. The inhomogeneous structure of ProRoot® MTA is remarkable

# 4. Discussion

In the literature, there are numerous articles focusing on the short- and long-term clinical results of different root-end filling materials. In addition, there have been also studies which have investigated the connective tissue response to the retrograde root-end filling materials, [32] neurotoxic behaviors [5] and radiological properties [13], however, a few studies have compared the physical properties of materials used as root-end fillings [39], [42], [43]. Current study aims to clarify the physical properties of five different canal sealers used as retrograde filling materials on the resected root apex following apicoectomies.

According to the SEM analysis results described herein, the marginal gap values of all tested materials ranged between 4.872 and 18.343 microns and were under 30 microns, which could be accepted within limits. The comparison of five materials showed that, Diaket™ achieved the best results compared to the other materials. The mean values of marginal gaps of AH-Plus™ and Super-EBA™ fast were about in the same magnitude, followed by Super-EBA™ Regular. However, the highest marginal gap value has ProRoot® MTA with 18.343 microns.

Diaket™ is one of the most studied material in endodontics as a retrograde root-end filling material. Lloyd et al. [22] investigated *in vitro* sealing ability of the root-canal sealer Diaket when used as a root-end filling material compared to of amalgam, using linear micro-leakage of Indian ink and showed that Diaket provided a superior sealing property compared to amalgam irrespective of the root-end preparation. Gerhards and Wagner [12] have investigated the sealing ability of amalgam, Harvard-Cement, Diaket, gold-leaf, and Ketac-Endo as retrograde root-end filling materials via stereomicroscope. According to their results, retrograde fillings with Ketac-Endo showed significantly less leakage compared with amalgam. There was no significant difference between the amalgam and Diaket subgroups. The sealing ability of Harvard-Cement and gold foil was lower than amalgam and they have concluded that retrograde fillings with Ketac-Endo or Diaket could be considered as alternative for amalgam.

In the literature, it has been proclaimed that microleakage could not be completely inhibited with amalgam, zinc oxide eugenol cement or glass-ionomer cement [4], [26], [28]. Based on results of different clinical and histological studies, ProRoot® MTA stands out as the gold standard retro-filling material for apical seal [12] due to its superior characteristics such as biocompatibility, non-toxicity, osteoinduction and cementogenesis [30], [31]. It has been suggested that MTA provides a very good seal, has excellent marginal adaptation, maintains a high pH for a long period, and appears to induce a favorable tissue response [40]. In addition, Maltezos et al. [23] compared the sealing properties of Resilon, ProRoot® MTA, and Super-EBA as root-end filling material and stated that ProRoot® MTA presents significantly less leakage than that with amalgam, gutta-percha and zinc oxide eugenol in a dye leakage test. Similarly, Tanaka et al. [38] reported that dye leakage of root-ends sealed without cavity preparation using 4-META/MMA-TBB resin was significantly lower than that of root-end fillings performed by using reinforced zinc oxide eugenol cement.

Surprisingly, the results of the *in vitro* studies focusing on the marginal properties of MTA are controversial. Wu et al [43] confirmed that ProRoot® MTA has a superior marginal adaptation compared to Super-EBA™. However, studies originating from different decades [1], [34], [41] could not confirm this property. Moreover, Adamo et al. [1] have proclaimed that microleakage of MTA has been reported to increase with time. Nabeel et al. [25] also highlighted the sealing ability of ProRoot® MTA. In the current study, the results obtained with ProRoot® MTA showed significantly higher gap values compared to other four materials. However, this statement warrants further evaluation.

The obturation properties and apical leakage of different sealers have been studied with a variety of experimental methods previously [33]. These are: dye penetration test, the use of radioactive isotopes, electrochemical analysis, penetration test with bacteria, air or water pressure, micro-radiography, scanning electron microscopy.

In the past, several studies [7], [44] have used SEM analysis in evaluation of marginal integrity of root end filling of various root canal material. In the current study, the quantification of the marginal gap analysis was performed by using SEM, whereas micro-radiography was used to help understanding of the qualitative examination. Similarly, the study of root canal fillings on marginal integrity was carried out as suggested by Möhlenkamp [25] and Spiekermann [38] with a combined approach for qualitative and quantitative examination of the apical leakage by SEM and complementary micro-radiography.

The negative point of SEM analysis is that the high vacuum could result in artificial cracks and crevices, which has been also observed during the current study [25]. Might one conclude that micro-cracks emerged during the SEM processing could negatively affect the quantification of the results presented herein. It is well known that hydration and drying processes of cement-like polymers could result in micro-shrinkage [39]. If this shrinkage exceeds the tensile strength of the material, micro-cracks could occur. Bisschop and van Mier [8] have proclaimed that the impregnation of the dried specimen prior to cross-sections could be reliable option to standardize drying shrinkage microcracks. Therefore, the technique described by Bisschop and van Mier [8] could be beneficial to overcome micro--crack related quantification problems in future studies.

One might suggest that filling of the root canals prior to retrograde sealing might change the results described herein. However, it should be kept in mind that the physical properties of different endodontic sealers could influence the polymerization process of the materials used for apical filling and might result artificial cracks regarding the amount of the vacuum required.

## 5. Conclusions

It is obvious that the retrograde obturation, which mostly depends on the marginal integrity of the root-end filling material, plays a great role in the success of the apicoectomy procedure. However, soft and hard tissue responses to the retrograde root-end filling material and alterations in the physical properties in the long term could also affect the clinical results of treatment.

According to the limited knowledge of the current *in vitro* study, it can be concluded that both in terms of its marginal integrity and material properties, Diaket™ is the most suitable material for retrograde obturation. Additional clinical studies with long term follow up period might help to determine the most appropriate root-end filling material.

## Author contributions

Conceptualization: Y.A. and A.G.; method: Y.A.; software: F.K. P.K.; validation: P.K., J.W.; SEM analysis, writing – original draft preparation: A.G. and M.E.; writing – review and editing: J.W.. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

The authors declare no conflict of interest.

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