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Research Article

Effect of Root Dentin Moisture on the Apical Sealing Ability of Root Canal Sealers: In vitro Study

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Abstract

Background: The goal of root canal filling is complete sealing of the canal system. The moisture level is crucial for effective sealing and adhesion. ***Objective***: To quantitatively evaluate the effect of root canal dentin moisture conditions on the apical sealing of two root canal sealers. ***Methods***: 54 single-rooted mandibular premolars were instrumented and randomly divided into two groups (n=27) according to the types of sealers used: group MTA Fillapex and group One-Fil bioceramic sealer. Then, both groups were randomly divided into 3 subgroups (n=9) according to different intracanal moisture conditions before obturation: dry (the canals were dried with paper points until the last one came out dry), moist (only one paper point was used), and wet (canals left wet without drying). After completing the obturation using sealers and gutta-percha, the apical leakage was evaluated by the dye extraction method using a spectrophotometer. ***Results***: The lowest leakage value was 0.006 for subgroup One-Fil, wet. While the highest leakage was 0.058 for subgroup MTA and dry. The statistical analyses revealed that One-Fil in wet conditions had significantly lower leakage than in moist and dry conditions. MTA in moist conditions had significantly lower leakage than in wet and dry conditions. There was a significantly lower value for One-Fil, wet, compared with MTA, wet, and MTA, moist compared with One-Fil, moist. ***Conclusions***: One-Fil sealer showed the lowest microleakage in wet conditions. While MTA Fillapex showed the lowest microleakage in moist conditions.

Keywords: Dentin moisture, Dye extraction, MTA Fillapex, One-Fil Bioceramic sealer.

تأثير رطوبة عاج الجذر على قدرة الختم القمي لسدادات قناة الجذر: دراسة في المختبر

الخلاصة

الخلفية: الهدف من ملء قناة الجذر هو الإغلاق الكامل لنظام القناة. مستوى الرطوبة أمر بالغ الأهمية للختم والالتصاق الفعالين. **الهدف**: التقييم الكمي لتأثير ظروف رطوبة عاج قناة الجذر على الختم القمي لاثنين من السدادات لقناة الجذر. **الطرائق:** تم تجهيز 54 ضواحك للفك السفلي أحادي الجذر وتقسيمها عشوائيا إلى مجموعتين (ن = 27) وفقا لأنواع السدادات المستخدمة: المجموعة MTA Fillapex والمجموعة One-Fil bioceramic sealer. بعد ذلك ، تم تقسيم كلتا المجموعتين بشكل عشوائي إلى 3 مجموعات فرعية (ن = 9) وفقا لظروف الرطوبة المختلفة داخل القناة قبل السداد: جافة (تم تجفيف القنوات بنقاط ورقية حتى خرجت الأخيرة جافة)، رطبة (تم استخدام نقطة واحدة فقط من الورق) ، ورطبة جدا (تركت القنوات مبللة دون تجفيف). بعد الانتهاء من السد باستخدام السدادات و gutta-percha، تم تقييم التسرب القمي بطريقة استخراج الصبغة باستخدام مقياس الطيف الضوئي. **النتائج**: كانت أقل قيمة تسرب 0.006 للمجموعة الفرعية One-Fil ، الرطبة جدا. بينما كان أعلى تسرب 0.058 للمجموعة الفرعية والجافة MTA. كشفت التحليلات الإحصائية أن One-Fil في الظروف الرطبة جدا كان لديه تسرب أقل بكثير مما هو عليه في الظروف الرطبة والجافة. كان لدى MTA في الظروف الرطبة تسرب أقل بكثير مما كان عليه في الظروف الرطبة جدا والجافة. كانت هناك قيمة أقل بكثير لـ One-Fil، رطبة جدا، مقارنة ب MTA، رطبة جدا، و MTA، ورطبة مقارنة ب One-Fil، رطبة. **الاستنتاجات**: أظهر مانع التسرب One-Fil أقل تسرب دقيق في الظروف الرطبة جدا. بينما أظهر MTA Fillapex أقل تسرب دقيق في الظروف الرطبة.

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INTRODUCTION

Successful endodontic treatment relies on thoroughly removing the infected pulp, properly cleaning the root canal, and ensuring appropriate biomechanical preparation and filling [1]. Most treatment failures resulted from inadequate obturation, with insufficiently filled gaps that might serve as a breeding ground for bacteria. The primary goal of endodontic obturation is to achieve a hermetic seal [2]. The adaptability of the root canal filling material to the canal walls is critical for the long-term success of the treatment. Gutta-percha and sealer should be used to fill the canal with a consistent mass that conforms to the canal walls. Without a sealer, the GP cannot properly adapt to the canal, leading to gaps [3]. There are different types of endodontic sealants; one of them is MTA Fillapex (Angelus, Londrina-Parana, Brazil), which was introduced to the market in 2010. This sealer contains MTA, bismuth oxide, salicylate resins, pigments, and silica nanoparticles in a two-paste system. Known for its high biocompatibility, bactericidal properties, and low solubility [4]. According to the manufacturer, MTA Fillapex's setting reaction is not a polymerization reaction but rather a complexation reaction made easier by the presence of moisture in the root canal. One-Fil bioceramic sealer (MEDICLUS, Cheongju, Korea) is a new bioceramic root canal sealer composed of

calcium aluminosilicate, a thickening agent, and zirconium oxide [5]. When hydraulic calcium silicate-based sealers come in contact with fluids in the environment, they harden. This makes calcium minerals that make the sealers more biocompatible and active [6]. However, as a newly developed product, One-Fil sealer has certain limitations due to the limited research available. The amount of water inside the root canal is not always the same after cleaning and shaping. It can be very different depending on the root's shape, the number and size of exposed dentinal tubules, and the root canal dryness protocols [6]. Variations in residual moisture levels within the root canal have been demonstrated to affect the sealing properties and adhesion of root canal materials [7]. Clinicians have varying views on moisture, as manufacturers recommend keeping the canal moist to benefit from the hydrophilic properties of the sealer. However, no standardization for moisture control after root canal irrigation has been precisely described. Several studies have shown that adhesion of sealers to root dentin [7-12] and apical sealing ability [13,14] can be affected by the moisture condition. However, there is still limited research to evaluate the apical sealing ability for One-Fil Bioceramic sealer and MTA Fillapex in different moisture conditions. Consequently, this study aimed to assess how root canal moisture conditions affect the apical sealing capacity of two types of sealers: One-Fil and MTA Fillapex. Hence, the null hypotheses of this study were 1) the apical sealing of One-Fil and MTA Fillapex would not be influenced by the different moisture conditions of root dentin. 2) The sealing ability of both sealers is the same.

METHODS

Study design and sample selection

Fifty-four single-rooted human premolars from patients aged 18-25 years old extracted for orthodontic reasons were selected. The study only included teeth with a single root, closed apex, and a single canal. Teeth with root resorption, caries, cracks, dental anomalies, fractures, or curved roots were not included. To make sure there was only one root canal, a radiograph was taken. A sharp periodontal curette (Medesey, Italy) was used for the removal of any remnants of soft tissue on the root surface. After that, all teeth remained in 0.1% thymol solution (DBH, England) for 24 hours and then in distilled water they changed every day until use [15]. The roots were marked by a marker pen at 13 mm [16,17] then fixed on a bench vice and sectioned perpendicular to the long axis of the root using a diamond disc bur (Horico Dental, Germany) with a straight handpiece and water coolant [18]. A barbed broach was used to remove the pulpal tissue. The patency was verified using a manual #10 K-file (Dentsply Maillefer, Switzerland) until it was observed at the apical foramen. By subtracting 1mm from the root length, the appropriate working length was determined [11,12]. The Endostar E3 Rotary File System (Endostar E3, Poldent, Poland) with a speed of 300 rpm and torque of 3.0 Ncm was used starting with a 30/08 rotary file, then 25/06, 30/04, 35/04, and 40/04 subsequently. 1 ml of NaOCl (Aqua, Turkey) was used to irrigate the canals prior to instrumentation. Subsequently, 1.0 milliliter of an NaOCl solution with a concentration of 2.5% was administered between each file size. A syringe with a 30-G side-vented needle was used to deliver the irrigation solution, positioned 2 mm short of the working length. Following the instrumentation, the canals were irrigated with 2 ml of 2.5% NaOCl [19]. Following the administration of 1 ml of EDTA (Cerkamed, Poland) for 1 minute, irrigation with 3 ml of 2.5% NaOCl and 5 ml of distilled water took place [20]. The 54 roots were randomly divided into 2 groups (n=27) according to the types of sealers used: group MTA-Fillapex (Angelus, Londrina-Parana, Brazil) and group One-Fil bioceramic sealer (MEDICLUS, Cheongju, Korea). Then, each group was randomly divided into three subgroups (n=9) according to moisture conditions before obturation: Dry: Paper points were used until the last one was dry [20,21]. Moist: One paper point was used to dry the root canals for 5 seconds [21]. Wet: the root canals were not dried and were left wet with distilled water [20,21]. The obturation was done according to the instructions from the manufacturer. MTA Fillapex is available as an auto-mix dual syringe. The auto-mixed sealer was dispensed on a paper pad, and the master cone (40/04) was coated with it, and obturation was completed using the single-cone technique. One-Fil BC sealer is premixed and injected into canals using the manufacturer's intracanal tip. For the single cone obturation technique, size 40/04 gutta-percha was used. After getting rid of the extra GP with a hot instrument 1 mm below the access hole, glass ionomer cement was used to seal the samples around the top. Radiographs were taken to assess the quality of the obturation. Subsequently, the samples were maintained at 37°C in an incubator (Memmert, Schwabach, Germany) for seven days to facilitate the complete setting of the sealers [11].

Dye extraction evaluation

For the dye evaluation test, 8 samples from each subgroup were selected for the apical seal evaluation by the dye extraction method. The samples were immersed in a neutralized buffer containing 2% methylene blue solution (BDH, UK) at 37°C for 24 hours under normal atmospheric pressure. Then, the teeth were washed with water for half an hour. Using a surgical blade and a polishing disc, the varnish coats were removed [22] (Figure 1).

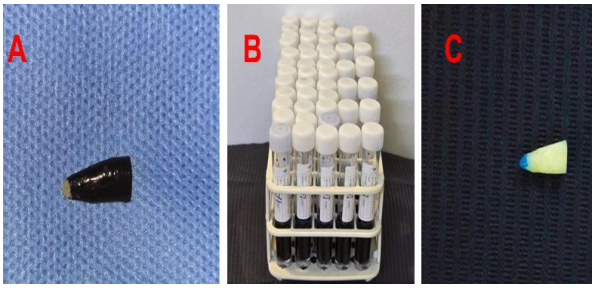

Figure 1: Samples preparation for dye extraction method; **A**) Root coated with two layers of nail varnish with the exception of apical 0.5 mm, **B**) The samples dipped for 24 h at 37°C in a neutralized buffer 2% methylene blue solution, **C**) Varnish removal.

After that, the samples were kept for three days in a container with 4 ml of 65% nitric acid. The solution is subsequently centrifuged at 4,000 rpm for 7 minutes in a Sigma 3-16L centrifuge (Osterode, Germany). Then, 2 ml of the supernatant layer from each sample was transferred to a plastic cuvette. Using concentrated nitric acid as a blank, the optical density of the solution was measured at 550 nm using an automated spectrophotometer (Emclub, Hanau, Germany) [22] (Figure 2).

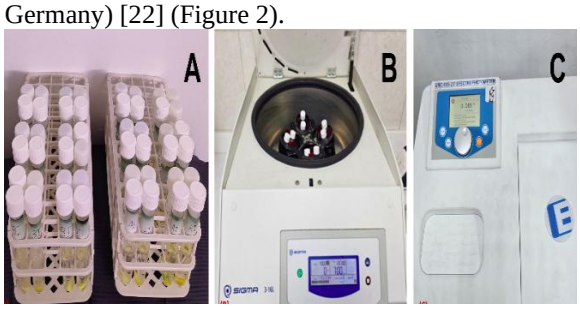

Figure 2: Dye extraction method; **A**) Samples in a container containing 4 ml of 65% nitric acid for 3 days; **B**) Centrifugation at 4000 rpm for 7 min; **C**) Spectrophotometer device for reading the optical density of the dye that penetrate samples.

Scan election microscopy (SEM)

The ninth sample that was left from each subgroup was used for SEM evaluation. After complete root instrumentation and obturation, the samples were embedded in self-curing transparent acrylic and sectioned perpendicularly to the long axis of the root canal at 2 mm from the anatomical apex. These sections were examined by SEM at 2000x magnification (Figure 3).

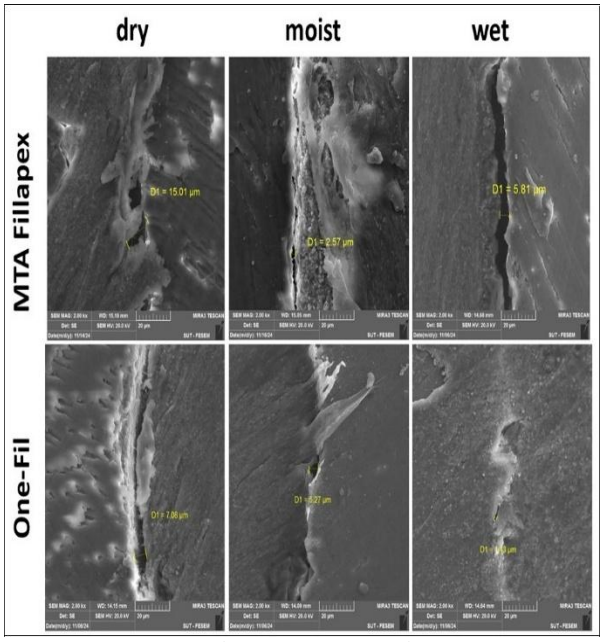

Figure 3: SEM images of the apical sections of the roots obturated with MTA Fillapex and One-Fil in dry, moist and wet conditions at x2000. (D1= Gap width).

Ethical considerations

The Local Research Ethics Committee of the College of Dentistry, at the College of Dentistry, Mustansiriyah University approved the study protocol (Certificate Number and Date: REC130 on 1/05/2023).

Statistical analysis

SPSS, version 26.0 (IBM Corporation, Armonk, NY, USA), was employed to conduct the data analysis. The Shapiro-Wilk test was employed to assess the normal distributions of the results. The hypothesis test was conducted using a one-way ANOVA, and pairwise comparisons were conducted using the Tukey HSD and Student's t-test.

RESULTS

Descriptive statistics, including mean, standard deviation, minimum, and maximum, of the spectrophotometric values of the optical density of the dye that leaked into the samples of each subgroup are shown in Table 1.

Table 1: Descriptive statistics of the dye leakage in different experimental groups.

Groups		No. of Samples	Mean±SD	Min-Max
One- Fil	Dry	8	0.057±0.016	0.03-0.08
	Moist	8	0.051±0.019	0.03-0.085
	Wet	8	0.006±0.004	0.001-0.011
MTA Fillapex	Dry	8	0.058±0.001	0.047-0.073
	Moist	8	0.022±0.009	0.01-0.036
	Wet	8	0.051±0.004	0.046-0.058

The lowest mean value was in subgroup One-Fil, wet (0.006), while the highest mean value was for subgroup MTA, dry (0.058). One-way ANOVA test revealed a significant difference among the experimental subgroups for each group (p=0.000). Tukey HSD showed a significant difference that was recorded for subgroup One-Fil, wet, compared to both One-Fil, moist, and One-Fil, dry (p< 0.0001). There was no significant difference between subgroup One-Fil, dry, and One-Fil, moist (p= 0.736). Subgroup MTA, moist exhibited a statistically significant difference compared to both MTA, wet and MTA, dry (p< 0.0001). There was no significant difference between the subgroups MTA, dry, and MTA, wet (p= 0.32) (Table 2). Based on the Student's t-test (Table 3), there was a significant difference between subgroups One-Fil, wet, and MTA, wet (p< 0.0001), and subgroups One-Fil, moist, and MTA, moist (p< 0.0001). SEM images for the three subgroups of MTA and One-Fil are represented in Figures 3. Smaller gaps were seen with the wet subgroup of both One-Fil and the moist subgroup of MTA. The largest gap between the sealer and root canal wall was within subgroup MTA, dry (15.01 µm), while the smallest was within One-Fil, wet (1.43 µm).

DISCUSSION

Failure in root canal treatment is highly related to the reinfection of the periapical area. The primary objective of root canal obturation is to effectively seal the root canal system to prevent reinfection. The presence of moisture and liquids within the root canal can adversely affect the sealing capability of any obturating material, as they may delay the setting reaction of the sealers or potentially enhance it. These influences may lead to an increase in leakage [13].

Table 2: Tukey HSD between each two groups

Subgroups	Subgroups	Mean difference±SE	*p*-value	95% CI
One-Fil, dry	One-Fil, moist	0.005±0.007	0.736	-0.013-0.023
	One-Fil, wet	0.05±0.007	<0.0001	0.032-0.068
One-Fil, moist	One-Fil, wet	0.05±0.007	<0.0001	0.027-0.063
MTA Fillapex, dry	MTA Fillapex, wet	0.006±0.004	0.324	-0.004-0.017
	MTA Fillapex, moist	0.036±0.004	<0.0001	0.025-0.046
MTA Fillapex, moist	MTA Fillapex, wet	0.029±0.004	<0.0001	0.019-0.04

Results were expressed as mean±SE.

Table 3: Student t-test for comparison of each moisture condition with both types of sealers

Groups	*p*-value
One-Fil, dry *vs*. MTA Fillapex, dry	0.88
One-Fil, moist *vs*. MTA Fillapex, moist	0.001
One-Fil, wet *vs*. MTA Fillapex, wet	<0.0001

However, no specific protocol for dryness of the root canal after mechanical instrumentation and irrigation was prescribed. The drying process used before root canal obturation might impact how well the sealer sticks to the dentin, the amount of microleakage, and the success of endodontic therapy [20]. Besides, different sealers with their different properties, according to their manufacturers' instructions, need specific handling concerning the root dentin wetness before application. Leakage evaluation in endodontics was done using a variety of methods. The dye extraction dissolution method is one of these methods in which it depends on calculating the amount of absorbed dye after dissolving the tooth substrate in acid. The optical density of the dye is quantified by a spectrophotometer. This method has been utilized in endodontic studies for years since it produces quantitative results, requires simple materials, and takes into account all absorbed dye in the samples. Also, this technique minimizes human measurement error and provides assessments of volume leakage instead of linear measurements, such as the dye penetration method [23]. The results of this study demonstrated that the moisture condition of root canals had a significant effect on the apical sealing ability of both sealers. Therefore, the null hypothesis was rejected. When considering One-Fil sealer independently, it recorded the lowest mean value of microleakage in wet conditions and a significant difference with dry and moist conditions. This result can be attributed to the fact that calcium silicate-based sealers require moisture for proper setting. When calcium silicate, the main component of these sealers, absorbs moisture from the root canal, it forms calcium hydroxide crystals. It is well known that the calcium hydroxide in the sealer absorbs water, making it expand. This effectively closes the space between the gutta-percha and the dentin in the root canal [24]. While in dry conditions, excessive desiccation may remove the water that is present in the dentinal tubules, which in turn hinders the ability of hydrophilic sealers to effectively seal [20,25]. One-Fil is a novel material that is marketed as a calcium aluminosilicate based bioceramic sealer [5]. The influence of moisture conditions on the apical sealing capability of this material remains unclear, and this finding cannot be compared with any other research. Previous studies [11,12,21] evaluated the effect of different drying protocols on the bond strength of different brands of bioceramic sealers (Sealer Plus BC, Bio-C Sealer, iRoot SP, and Endosequence BC sealer) and concluded that the best result was achieved when the canals were dried by paper points until the last paper point came out totally dry. While A study by Taşdemir *et al*. [8] demonstrated that bioceramic sealer (iRoot SP) had a greater bond strength when the canals were dried with a single paper point. The good bond strength between obturation materials and dentin indicates less microleakage, which is crucial for the success of endodontic procedures [26-28]. In the present research, MTA Fillapex demonstrated the lowest mean value of microleakage in moist conditions and a significant difference with dry and wet conditions. This result is consistent with a previous study that indicated that the retention characteristics of MTA could be significantly influenced by curing conditions. The dry-cured MTA exhibited a lower push-out bond strength to dentin than the wet-cured MTA [29]. The chemical reactions of MTA Fillapex could support this outcome. The two-chemical reaction of this sealer involves hydration of calcium silicates, mainly tricalcium silicate and dicalcium silicate, when MTA Fillapex comes into contact with water [30]. These reactions produced calcium silicate hydrate gel and calcium hydroxide. Over time, C-S-H gel continues to polymerize, creating a nanostructured matrix that enhances the mechanical properties of the sealer. The final reaction occurs between salicylate resin and calcium hydroxide to form calcium chelates, improving its workability and flowability [31]. Therefore, the presence of water may have enhanced the chemical reactions between the sealer components. The results of this study contrasted those of other studies [32,33], in which moisture conditions did not influence the sealing efficacy of MTA Fillapex at the apical region of the root canal. The variance in the results may be attributed to the differences in the type and storage of the teeth, as well as the methodologies employed. However, Ozlek *et al*. [20], when investigating the effects of dentin moisture conditions on the push-out bond strength of MTA Fillapex and GuttaFlow BioSeal, comparing the dentin moisture conditions as dry (paper points until the last paper points came out totally dry), moist (using EndoVac then one paper point), and wet (the root canals were left wet with distilled water), showed that the highest bond values were observed in the EndoVac group. While another study, which tested the effect of moisture on dentin bond strength of AH Plus, Sealapex, and MTA Fillapex, concluded that lower bond strength values for the groups that were dried with EndoVac [33]. The results of the current study demonstrated that One-Fil had a significantly lower mean microleakage compared to MTA Fillapex in wet conditions. This result is consistent with the findings

of Nagas *et al*., who observed that MTA Fillapex exhibited lower bond strength values compared to bioceramic sealer [7]. This result was attributed to the different composition of these sealers. MTA Fillapex contains salicylate resin in its composition, which may contribute to polymerization shrinkage [34]. Thus, higher amounts of salicylate resin and long setting times may cause dimensional changes and gaps between root canal walls and filling materials. This effect may affect root canal sealer bond strength directly and indirectly by affecting flowability and solubility, which are also important for dentinal wall sealing [30]. MTA Fillapex comprises less than 20% MTA particles, making it inadequate to exhibit the complete biological and sealing properties of this cement [35]. Moreover, MTA Fillapex contains bismuth oxide as the radiopacifier, which is associated with a decrease in mechanical strength, increased porosity, and material degradation. One-Fil, on the other hand, does not contain resin in its composition, making it stable over time. In addition, it exhibited a significantly greater flow compared to other bioceramic sealers. Flow is a crucial characteristic in root canal filling, as it directly influences the sealer's ability to penetrate the root canal system effectively [5]. Another feature that calcium silicate-based sealers exhibit is chemical bonding to the dentin walls of root canals. CSBS forms a specific interfacial layer at the dentin walls known as the mineral infiltration zone [5]. This zone is characterized by the appearance of tag-like structures of calcium silicate at the site of interference between calcium silicate and dentin [36]. Calcite crystals are formed when the calcium ion that is present in this zone combines with the carbon dioxide that is present in the tissue. Using these crystals leads to decreased gaps and leakage and improved adaptation [37,38].

Study Limitations

There are some limitations on this study. Since it was conducted in a lab, it does not accurately represent actual clinical settings. Only straight, single-rooted teeth were included in the small sample size, which may not accurately reflect other root canal varieties. Furthermore, the long-term effects are unknown because the dye extraction method was only used to examine the sealing ability for a brief period of time. Lastly, because One-Fil BC is a recently developed sealer with little research data, it is more challenging to evaluate the results, as there are few prior studies for comparison. More studies are still required to assess how moisture conditions affect its sealing and long-standing adaptation to the root canal walls.

Conclusion

Within the limitation of the current in vitro study, it can be concluded that MTA Fillapex sealer showed less microleakage in moist conditions. One-Fil bioceramic sealer demonstrated less microleakage in wet conditions. Microleakage in wet conditions was significantly lower than in dry and moist conditions for One-Fil BC sealer. Microleakage in moist conditions was significantly lower than in dry and wet conditions for MTA Fillapex sealer. Additionally, One-Fil in wet conditions resulted in significantly lower leakage than MTA Fillapex. While MTA Fillapex in moist conditions resulted in significantly lower leakage than One-Fil.

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Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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