

## Original Article

# Shear bond strength and scanning electron microscopy characteristics of universal adhesive in primary tooth dentin: An *in vitro* study

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## ABSTRACT

**Background:** The aim of the study was to evaluate the adhesion of Scotchbond Universal Adhesive to primary tooth dentin by measuring shear bond strength (SBS) and observing morphological changes with scanning electron microscopy (SEM).

**Materials and Methods:** In this *in vitro* study, a total of 60 primary canine teeth were randomly divided into 5 groups ( $n = 12$ ). The study groups were (1) Phosphoric acid etching + Adper Single Bond 2 (control), (2) phosphoric acid etching + Scotchbond Universal (etch-and-rinse), (3) Scotchbond Universal (self-etch), (4) phosphoric acid etching + Scotchbond Universal + resin, and (5) Scotchbond Universal + resin. Composite cylinders were built on the tooth surface, and 10 samples in each group were selected for SBS testing and identification of the failure modes. Two samples from each group were observed by SEM. One-way ANOVA and Tukey honestly significant difference *post hoc* test were used for data analysis.  $P$  value  $< 0.05$  was considered statistically significant.

**Results:** The results showed that SBS in Group 1 was significantly lower than in Groups 2, 3, and 4 (all  $P < 0.001$ ). There was no significant difference between Groups 2 and 3 ( $P = 0.98$ ), or between Groups 3 and 4 ( $P = 0.97$ ). There was no significant difference between Groups 2 and 4 ( $P = 0.999$ ) or between Groups 1 and 5 ( $P = 0.156$ ). Mixed and cohesive failures were more frequent in Groups 2, 3 and 4. SEM observations showed that applying phosphoric acid to the dentin before Scotchbond Universal adhesive resulted in more open dentinal tubules and more resin tag impregnation.

**Conclusion:** There was no significant difference in SBS between Scotchbond Universal Adhesive in etch-and-rinse and self-etching modes. The SBS of Scotchbond Universal Adhesive in etch-and-rinse mode was greater than Adper Single Bond 2.

**Key Words:** Adhesive, bond strength, primary teeth, scanning electron microscopy, universal

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## INTRODUCTION

For many years, dental adhesive systems have been used to enhance the adhesion between tooth structures and composite resins. Dental adhesives can be categorized according to the time of use (generation of calcification) or the method of use (etch-and-rinse or self-etch) and influence on the smear layer. Conventionally,

the systems include etch-and-rinse adhesives applied in three steps (fourth-generation) or two steps (fifth-generation or one-bottle adhesives).<sup>[1]</sup> These systems require phosphoric acid etching associated with a separate rinsing step (total-etch technique).

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To simplify clinical procedures, self-etch adhesives have been developed. Two-step self-etching adhesives (sixth generation) require applying an acidic primer and adhesive resin in two separate steps. The application of a separate primer is omitted in one-step self-etch adhesives (seventh generation) thanks to the use of a solution in one bottle containing an acidic primer and resin together. These materials are less clinically sensitive, require less operation time and are easy to use, which are all important in pediatric dentistry.<sup>[2]</sup>

During 2011–2012, a new generation of one-bottle adhesives called “universal”, “multi-mode” or “multi-purpose” adhesives were introduced. The manufacturers recommend using the adhesive systems with two methods: (1) as an etch-and-rinse adhesive with selective acid etching before the adhesive is applied, or (2) as a one-bottle self-etching adhesive without additional etching.<sup>[3-6]</sup> The clinical benefits of universal adhesives (UA) such as Scotchbond Universal Adhesive (SBU) include ease of application and reduced patient chair time (advantageous especially in children); however, the methods used to apply the UA are different and clinicians need to be aware of these differences.<sup>[3,5]</sup>

Some researchers compared the sealing ability and bond strength of the two approaches using a UA for composite resin restorations in permanent teeth. Most studies showed that using phosphoric acid etching enhanced the microtensile bond strength ( $\mu$ TBS) of the UA to permanent enamel, especially when a mild adhesive was used.<sup>[3,6-9]</sup> In this case, accidental etching of the dentin may occur during selective enamel etching despite appropriate precautions by the clinician. Several studies focused on the effect of permanent tooth dentin pre-etching before using the UA.<sup>[3,6,10]</sup> However, the effect of these two modes on permanent dentin remains somewhat controversial. One research found that shear bond strength (SBS) of the UA in permanent dentin decreased following preetching compared to samples without etching; however, the difference was not significant and depended on the adhesive material.<sup>[10]</sup> One study reported that preetching of permanent dentin improved penetration of the adhesive and noted that both UA etching modes (self-etch or total-etch) were reliable for dentin under clinical conditions.<sup>[11]</sup> Another studies reported that using a UA in a self-etch approach improved  $\mu$ TBS to permanent dentin and found no significant difference in immediate bond strength of the UA when used in total-etch or self-etch

techniques.<sup>[11-14]</sup> Some researchers recommend using a separate resin layer after the application of a UA to increase bond strength.<sup>[15,16]</sup>

Few studies have investigated the performance of UAs in primary teeth. One study showed that there was no significant difference between etch-and-rinse and self-etch modes when SBU was used in primary dentin,<sup>[10]</sup> where as another concluded that the etch-and-rinse approach was preferable before using a UA to improve  $\mu$ TBS in primary dentin.<sup>[17]</sup> Other work found that saliva contamination did not influence the SBS of SBU in primary or permanent tooth dentin.<sup>[18]</sup>

Because few data are available on the use of UAs in primary teeth, the purpose of this *in vitro* study was to determine the effectiveness of a new one-step self-etch adhesive in primary tooth dentin. The adhesive was applied with two different methods: selective preetching (etch-and-rinse) and self-etching. The two methods were compared for SBS and scanning electron microscopic (SEM) morphological characteristics.

## MATERIALS AND METHODS

The protocol of this *in vitro* study was approved by the Human Ethics Review Committee of the School of Dentistry, Shiraz University of Medical Sciences (grant number# 99-10169). A total of 70 primary canine teeth extracted due to orthodontic treatment were cleaned and stored in 0.1% chloramine T solution for 4 weeks for disinfection. The aim of the study was explained to the parents, who provided their informed consent in writing. Next, the teeth were immersed in distilled water at 37°C. The tooth surfaces were examined under a stereomicroscope to rule out teeth with cracks, abrasions or caries. Finally, 60 sound teeth were selected, and the root of each tooth was sectioned transversally 2 mm below the cemento-enamel junction with a water-cooled diamond saw.

### Dentin block preparation

The teeth were mounted in acrylic resin with the tooth long axis perpendicular to the upper surface of the mold. Then, about one-third of the incisal edge of the occlusal surface of tooth was cut and flattened with a diamond wheel bur to provide a bonding area in midcoronal dentin. The dentin surface was smoothed with 600-grit silicon-carbide paper for 1 min under water cooling to standardize the smear layer. After ultrasonic cleaning, rinsing and

drying, all specimens were carefully checked under a stereomicroscope (Motic K, Wetzlar, Germany) to verify that the dentin surface was intact without pulp exposure.<sup>[3]</sup>

The teeth were stored in wet conditions for 24 h. Then, the samples were randomly divided into five groups containing 12 teeth each, in which dentin pretreatment was performed before SBS was measured.

- Group 1 (control): Phosphoric acid etching (3M, ESPE, St. Paul, MN, USA) +Adper Single Bond 2 (3M, ESPE)
- Group 2: Phosphoric acid etching + Scotchbond Universal Adhesive(3M, ESPE)(etch-and-rinse)
- Group 3: Scotchbond Universal Adhesive (self-etching)
- Group 4: Phosphoric acid etch + Scotchbond Universal Adhesive + resin layer
- Group 5: Scotchbond Universal Adhesive + resin layer.

The composition of the materials and instructions for use are shown in Table 1.

After the adhesive was applied, 10 samples in each group were selected for SBS testing and identification of the failure modes. A rubber cylindrical mold 3 mm in internal diameter and 3 mm in height was used to bond the composite resin to the dentin. The cylinder was filled with composite resin (Z250, 3M, ESPE, St Paul, MN, USA) with an incremental technique. Each 1.5 mm layer was polymerized for 40 s with a halogen light-curing unit (Coltolux, Coltène/Whaledent AG, Altstätten, Switzerland) at a power density of 550 mW/cm<sup>2</sup>. Then,

the specimens were stored in humid conditions at 37°C for 24 h. SBS was measured with a universal testing machine (Zwick-Roell, Zwick, Ulm, Germany). Shear load was applied using a knife-edge blade at a crosshead speed of 1 mm/min until failure occurred. The peak load at failure was recorded and divided by adhesive surface area to obtain SBS in megapascals (MPa). The type of bond failure of the fracture was evaluated by two observers under blind conditions with a digital microscope (Dino Lite, Taipei, Taiwan) at ×25 magnification and was recorded as follows:

- Adhesive fracture at the composite-dentin interface
- Cohesive fracture in the substrate, that is, dentin
- Mixed fracture with both adhesive and cohesive fracturing.<sup>[19]</sup>

### Scanning electron microscopy observation

Two prepared samples were selected from teeth in each experimental group for SEM evaluation. The specimens were sectioned perpendicular to the adhesive interface and polished with 400, 600, 1000, and 2000 grit silicon-carbide papers under water cooling. The teeth were rinsed, and the sectioned surfaces were treated with 37% phosphoric acid for 10 s, rinsed for 30 s, and immersed in 5% NaOCl for 2 min. After rinsing, the specimens were dehydrated in a series of 70%, 80%, 90%, and 99% ethanol. Then, the samples were sputter coated with gold in a vacuum evaporator. Micromorphological changes were examined in a scanning electron microscope (KYKY-EM3200, Shanghai, China) at ×500 and ×1500 magnification.

**Table 1: Materials and application procedures used in this study**

Materials	Chemical composition	Procedures	Manufacturer
Phosphoric acid gel	Phosphoric acid gel (37%)	Apply for 15 s, rinse 15 s, air-dry for 10 s	3M ESPE, St. Paul, MN, USA
Adper Single Bond 2	Primer: Bis-GMA, HEMA, Adhesive: Water, ethanol, polyalkenoic acid copolymer, photoinitiator	Apply and leave for 20 s, dry gently 2 to 5 s, light cure for 20 s	3M ESPE, St. Paul, MN, USA
Scotchbond Universal	10-MDP phosphate monomer, methacrylate modified polyalkenoic acid copolymer filler, HEMA, dimethacrylate resins filler, silane, initiators, ethanol, water	Self-etch strategy: Apply the adhesive, rub for 20 s. Rewet the disposable applicator during treatment. Gently air dry for 5 s, light cure for 10 s Etch and rinse strategy: Apply etchant for 15 s, rinse for 10 s, air dry for 2 s, apply adhesive as described for self-etch	3M ESPE, St. Paul, MN, USA
Resin	Unfilled resin		Resist, BiodentalTechnologies, Sydney, Australia
Composite resin Z250	Inorganic filler: Zirconium/silica with a particle size range of 0.01 to 3.5 µm. Organic matrix: Bis-GMA, UDMA, Bis-EMA	Fill molds with incremental technique	3M-ESPE, St Paul, MN, USA

Bis-GMA: Bisphenol A glycidyl methacrylate; HEMA: Hydroxyethyl methacrylate; UDMA: Urethane dimethacrylate; Bis-EMA: Bisphenol A polyethylene glycol dimethacrylate; MDP: Methacryloyloxydecyl dihydrogen phosphate

## Statistical analysis

One-way ANOVA was used to compare the mean SBS in different groups. Multiple comparison analyses were done with the Tukey honestly significant difference *post hoc* test. Before ANOVA, the normality assumption was verified with Kolmogorov–Smirnov test, which showed that the distribution of values for the SBS variable is normal in all groups (all  $P > 0.05$ ).  $P$  value  $< 0.05$  was considered statistically significant.

## RESULTS

There were significant differences in SBS between all five groups ( $P < 0.001$ ). Pairwise comparisons showed that mean SBS in Group 1 (control) was significantly lower than in groups 2, 3, and 4 (all  $P < 0.001$ ). There was no significant difference between Groups 2 and 3 ( $P = 0.98$ ) or between Groups 3 and 4 ( $P = 0.97$ ). There was no significant difference between Groups 2 and 4 ( $P = 0.999$ ) or between Groups 1 and 5 ( $P = 0.156$ ). Mean SBS in Group 5 was significantly lower than in Group 2 ( $P = 0.025$ ) and Group 4 ( $P = 0.029$ ). However, there was no significant difference between Group 5 and Group 3 ( $P = 0.131$ ). Table 2 shows the mean SBS values for all groups.

**Table 2: Mean shear bond strength (unit=MPa; n=10) after different surface pretreatments in primary tooth dentin**

Group	Mean±SD	P*
1. Phosphoric acid etch + Adper Single Bond 2	11.43±1.86 <sup>A</sup>	<0.001
2. Phosphoric acid etch + Scotchbond Universal (etch and rinse)	17.55±2.18 <sup>B</sup>	
3. Scotchbond Universal (self-etch)	16.90±2.64 <sup>B,C</sup>	
4. Phosphoric acid etch + Scotchbond Universal + resin	17.58±1.89 <sup>B</sup>	
5. Scotchbond Universal + resin	14.04±3.39 <sup>A,C</sup>	

\*One-way ANOVA  $F$ -test. Mean SBS values with the same capital letters in superscript were not significantly different (Tukey HSD test). SBS: Shear bond strength; HSD: Honest significant difference; SD: Standard deviation

**Table 3: Frequency of failure modes of fractures after different surface pretreatments in primary tooth dentin (unit=N)**

Groups	Failure mode		
	Adhesive (at dentin/composite interface)	Cohesive (at dentin)	Mixed (both adhesive and cohesive fracture)
1. Phosphoric acid etch + Adper Single Bond 2	6	1	3
2. Phosphoric acid etch + Scotchbond Universal (etch-and-rinse)	1	3	6
3. Scotchbond Universal (self-etch)	2	4	4
4. Phosphoric acid etch + Scotchbond Universal + resin	1	4	5
5. Scotchbond Universal + resin	5	2	3

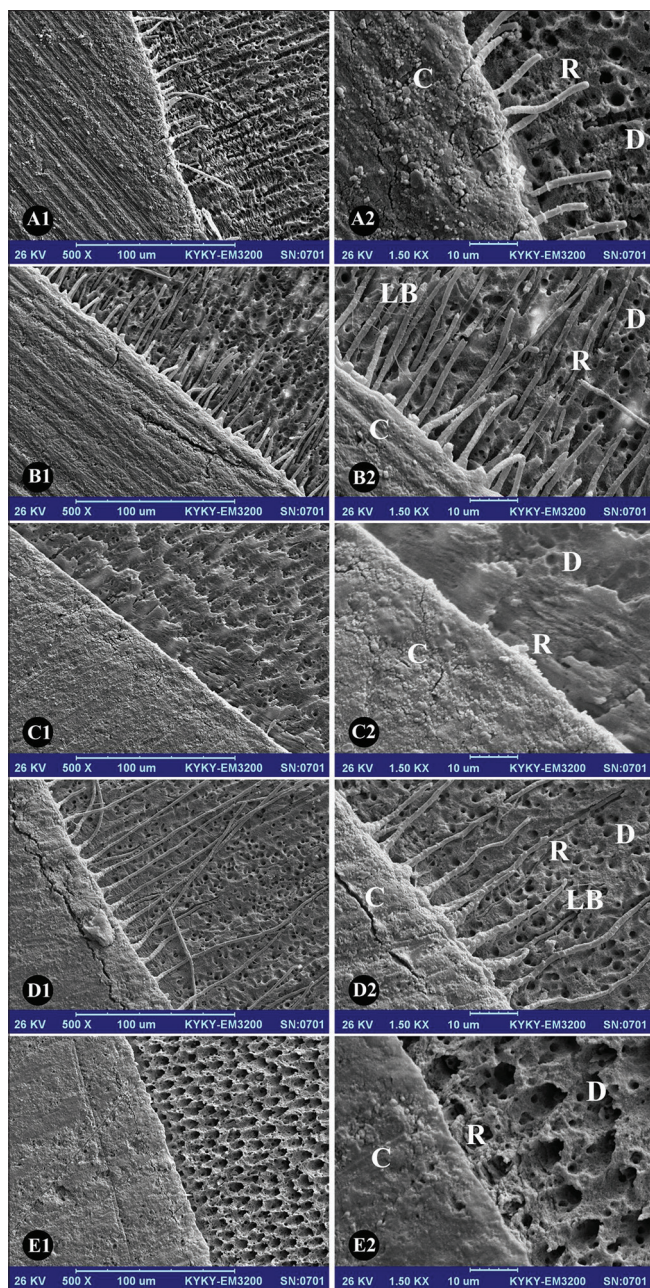
The results for failure mode showed that cohesive and mixed fractures were the most frequent fracture modes in Groups 2, 3, and 4. Table 3 shows the frequencies of different failure modes in all groups.

## Scanning electron microscopy evaluation

All samples in which dentin was pretreated with phosphoric acid etching had the same micromorphological characteristics. Acid etching led to the removal of more minerals, with more open dentinal tubules and an irregular surface [Figure 1 A1, B1 and D1]. After the application of Adper Single Bond 2 on pretreated dentin, areas impregnated with moderate amounts of resin were observed beyond the hybrid layer [Figure 1 A2]. However, after SBU was applied, more resin tag impregnation was observed in most areas of the conditioned dentin. The resin tags were uniform, compact, and oriented in different directions [Figure 1 B2]. The effect of SBU in self-etch mode is shown in Figure 1 C1 and C2. Fewer open tubules were seen compared to pretreatment with phosphoric acid, due to the slight roughness of the dentin. Furthermore, less resin impregnation was observed than when SBU was used in etch-and-rinse mode [Figure 1 B1 and B2]. The application of a hydrophobic resin layer after the UA did not change the micromorphological characteristics of the interface. Long-resin tags with lateral branches were seen [Figure 1 D1 and D2] whereas the application of an additional resin layer did not influence resin infiltration into dentin that was pretreated with SBU in self-etch mode, and few resin tags were observed [Figure 1 E1 and E2 ].

## DISCUSSION

We investigated the effect of prior phosphoric-acid etching on the SBS of a multimode adhesive in primary tooth due to differences between primary and permanent dentin, which the former is more reactive to acidic conditioner. Our results can help guide



**Figure 1:** Scanning electron microscopy images of primary tooth dentin: phosphoric acid etching and application of Adper Single Bond 2 (A1 and A2), phosphoric acid etching and application of Scotchbond Universal adhesive alone (B1 and B2), Scotchbond Universal adhesive with self-etching (C1 and C2), phosphoric acid etching and Scotchbond Universal adhesive with a resin layer (D1 and D2), or Scotchbond Universal adhesive (self-etching) with a resin layer (E1 and E2) (500 $\times$ , 1500 $\times$ ). Dentin (D), Composite resin (C), Resin tag (R), LB (Lateral branches).

pediatric dentists who need to decide whether to use a UA in etch-and-rinse or self-etch modes.

Our data showed that the SBU system in both etch-and-rinse and self-etch modes led to greater

SBS than the use of a two-step etch-and-rinse bonding agent (Adper Single Bond) commonly used by dentists. Some previous studies reported similar or better performance of the UA compared to the two-step etch-and-rinse bonding agent.<sup>[20,21]</sup> However, other studies reported less adhesion to the permanent dentin following the use of a UA than when other bonding agents were used.<sup>[15,10]</sup> Muñoz *et al.* (2014) showed that the performance of a UA in permanent tooth dentin depended on the method of application (etch-and-rinse or self-etching). They reported that Peak Universal Adhesive had a  $\mu$ TBS similar to dentin compared to an etch-and-rinse (Adper Single Bond) or a self-etching adhesive (Clearfil SE-Bond).<sup>[15]</sup> We chose Adper Single Bond for comparison because it uses the same technology as SBU. Both adhesives contain polyalkenoic acid to provide chemical bonding with hydroxyapatite in the dentin. In addition, SBU includes 10-MDP (methacryloyloxydecyl dihydrogen phosphate), which provides a stronger bond to tooth structures than Adper Single Bond.<sup>[5,6]</sup> 10-MDP is capable of forming nano-layers with calcium. The MDP-calcium complexes can facilitate to cross-link collagen fibrils in the hybrid layer and bridge formation between collagen in the hybrid layer and adhesive monomers in the adhesive layer.<sup>[1,22]</sup>

We found no significant difference in the SBS of SBU between self-etching and etch-and-rinse modes. This is in agreement with a previous study in sound permanent dentin.<sup>[3]</sup> Another study did not find significant differences between these two modes of application in artificially-induced dentin with caries.<sup>[23]</sup> Lenzi *et al.* reported that there was no significant difference between  $\mu$ TBS of two-step etch-and-rinse adhesive (Adper Single Bond 2) and SBU in primary dentin in both etch-and-rinse and self-etch modes during wet and dry conditions.<sup>[24]</sup> The lack of significant difference between SBS of SBU to permanent dentin in etch-and-rinse and self-etch modes was reported by a recent study.<sup>[25]</sup> However, some researchers reported that prior phosphoric-acid etching decreased the bond strength of the UA to dentin.<sup>[26]</sup> The differences between results may be due to the “immediate” measurement of bond strength in our study, the type of tooth substrate and the type of UA used.<sup>[3,26]</sup>

Scotchbond Universal Adhesive has low-technique sensitivity and is an ultra-mild self-adhesive (pH 2.7) that contains water and the hydrophilic monomer (hydroxyethyl

methacrylate).<sup>[27]</sup> This functional monomer improves tooth wettability and infiltration and prevents hydrophobic monomer/water phase separation.<sup>[28]</sup> Water is a critical component because it ionizes the acidic groups, allowing the formation of hydronium ions. These ions are responsible of etching ability of hydroxyapatite.<sup>[5]</sup> However, the presence of water may increase the amount of unreacted monomer and lead to the formation of a porous hybrid layer.<sup>[29]</sup> Because of this, one study recommend applying an extra resin coating after the use of a UA to increase the thickness and uniformity of the adhesive layer and reduce fluid flow across the adhesive interface.<sup>[30]</sup> In the present study, our comparison of Groups 2 and 4 showed that applying a hydrophobic resin layer did not significantly change the SBS of SBU in etch-and-rinse mode. However, the SBS of SBU in self-etching mode with a resin layer (Group 5) was lower than when SBU was used without the resin layer (Group 3). This result may be due to the creation of a thick layer over the tooth surface, which might have decreased bond strength. One study showed that the  $\mu$ TBS of SBU decreased with time regardless of whether a resin layer was used in etch-and-rinse mode. The same tendency was also observed for the self-etching mode without a resin layer.<sup>[31]</sup> The differences between the results may be due to differences in adhesive composition, the method used to apply the UA in etch-and-rinse and self-etching strategies, the type of tooth, and the method of evaluation used.

We used SEM images to observe morphological changes in the interface after different methods of dentin surface treatment. Our SEM findings were consistent with the SBS results in all experimental groups. Compared to the two-step total-etch adhesive method (Group 1), applying SBU in etch-and-rinse mode led to more resin tags and resin impregnations beyond the hybrid layer, and greater micromechanical interlocking, all of which influence bond strength. In self-etching mode, SBU led to the removal of minerals and partial exposure of the tubules. As a result, fewer resin impregnations were observed. Applying a hydrophobic resin layer did not change the surfaces prepared with SBU in either etch-and-rinse or self-etching modes, and these observations were consistent with our SBS data.

Both cohesive and mixed modes of failure were observed in SBU in both etch-and-rinse and self-etching modes, a result that agrees with previous studies. Scotchbond Universal Adhesive led to more adhesive failure when used in self-etching mode

together with resin. In this connection, an earlier study reported that higher bond strength leads to a higher number of cohesive fracture failures.<sup>[16]</sup>

In this study, we evaluated the SBS of a UA in primary dentin under laboratory conditions. This method has been used previously to assess material resistance to contraction and biting forces.<sup>[32]</sup> One of the limitations of the present study was the small sample size, which may have influenced the results. Another possible limitation is that *in vitro* studies do not reflect clinical conditions. In addition, only one type of UA was evaluated, and the results may differ for other types of UA with different chemical compositions. We, therefore, recommend additional clinical studies in larger groups of pediatric patients.

## CONCLUSION

The shear bond strength of Scotchbond Universal Adhesive in etch-and-rinse mode was greater than a two-step total-etch adhesive (Adper Single Bond 2). There was no significant difference in Scotchbond Universal Adhesive shear bond strength between etch-and-rinse and self-etching modes. Applying a hydrophobic resin layer did not increase the shear bond strength of Scotchbond Universal Adhesive to primary dentin.

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## Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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