



Microhardness of early enamel lesions in deciduous teeth treated with an infiltrant resin, a fluoride varnish and GC tooth mousse

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General Note



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ABSTRACT

Background and Aim: Early enamel caries in primary dentition are highly prevalent and conservative treatments are needed at this stage. Considering the structural differences between primary and permanent teeth, this study aimed to compare the microhardness of early enamel lesions of deciduous teeth, treated with an infiltrant resin, a fluoride varnish and GC Tooth Mousse. **Materials and Methods:** Thirty six intact primary canines were included in the present in-vitro study. They were immersed in a demineralizing solution and incubated at 37° C for 4 days. The samples were randomly assigned into three study groups (rein infiltrate (group 1), GC Tooth Mousse (group 2) and fluoride varnish (group 3); 12 samples within each) and differently treated. Microhardness was evaluated before and after the remineralization process and after treatments for each specimen using a Vickers hardness testing machine. Data analysis was performed using paired T test on SPSS v22 software. **Results:** In group 2, the microhardness coefficient was increased significantly ($P=0.04$) from 133.3 to 155.4 (+22.1 V). The microhardness coefficient for group 1 was increased, but not significantly, ($P=0.2$) from 139.7 to 152.4. In group 3, the microhardness coefficient was significantly ($P= 0.001$) increased from 138.6 to 178.3 (+39.7 V). **Conclusion:** The resin infiltrate enhanced the surface microhardness, but not significantly. The fluoride varnish and the tooth mousse both increase the surface microhardness of enamel by improving the remineralization process.

Keywords: infiltrant resin - fluoride varnish - GC Tooth Mousse

1. INTRODUCTION

With a multifactorial etiology, dental caries is the most common childhood chronic disease. It involves an interaction between the host, food substrate, and aciduric bacteria. The caries process is dynamic and continuous, resulting from many cycles of demineralization and remineralization of the tooth structure after exposure to dietary fermentable carbohydrates. The acid produced by the plaque cariogenic bacteria leads to dissolution of calcium and phosphorus from the hydroxyapatite crystals of the enamel, and subsequently induce microscopic porosity within the remaining tooth structure (Cury & Tenuta, 2009). The first evidence of demineralization at the enamel surface is the appearance of a white spot. Presence of porosity in the subsurface enamel causes the loss of natural translucency and produces a white chalky appearance (Subramaniam et al., 2005). Remineralization of the early subsurface lesions can occur if the surface layer of the enamel is intact. But if the demineralization progresses, the thin surface layer will break down and a cavitated lesion will be generated (Gugnani et al., 2012). The treatment of early enamel caries has been based on non-invasive preventive therapies such as oral hygiene instruction, diet improvement or the use of topical fluoride. However, if these principles are not followed by the patient, many lesions tend to progress and require second-level preventive interventional treatments, many of which necessitate routine restorative therapies requiring removal of a large amount of intact enamel (Paris et al., 2013). One of the common methods for preventive treatment is the use of topical fluoride such as a fluoride varnish. Most varnishes contain 5% sodium fluoride. They are applied to clean; dry tooth surfaces and set quickly. They enhance remineralization by forming fluorohydroxy apatite (Murakami et al., 2009). Another approach to manage early carious lesions is to use compounds consisting of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) which is richly found in GC Tooth Mousse paste. GC Tooth Mousse paste, made from milk casein, contains calcium and mineral phosphate, and has been found to be greatly effective in preventing dental caries (Walsh, 2010). One of the recently introduced less invasive methods (microinvasive) for treatment of early caries is the use of high-penetrating resins into the porosity of enamel caries, leading to increased microhardness and strength of the lesion. It reduces porosity and prevents the breakdown of the intact enamel structure (Taher et al., 2012). Several parameters have been studied about the infiltrant resin technique in early caries of primary and permanent dentition, including changes in microhardness and surface roughness of enamel, enamel wear and depth of penetration into the lesion following the application of the resin (Pancu et al., 2011; Paris et al., 2007). Behrouzi and co-workers evaluated the effects of two remineralizing agents (MI –Paste Plus, Remin Pro) and a resin infiltrate on the microhardness of early carious lesions and found that microhardness values of teeth treated with either MI –Paste Plus or Remin Pro were significantly increased while the application of the infiltrant resin did not change the microhardness significantly (Behrouzi et al., 2019). Yazkan and colleagues assessed the technique of infiltrant resin in early carious lesions. The results showed that the enamel treated with infiltrant resin and microabrasion had similar microhardness values with no significant difference compared to the sound enamel. Infiltrant resin treated teeth showed less surface roughness than the microabraded teeth. Subsequent demineralisation did not affect the hardness for ten days but increased the roughness of the infiltrated and micro-abraded enamels. Polishing did not affect the roughness of micro-abraded enamel surfaces

(Yazkan & Ermis, 2018). The results of the research by Dorii and workmates indicated that the infiltrant resin technique is significantly more effective than non-invasive therapies such as fluoride varnishes in the treatment of early non-cavitated carious lesions of the enamel and dentin (the lesions limited to the outer one-third of dentin in radiographies) (Dorii et al., 2015). Due to the high prevalence of early carious lesions in children, the need for conservative treatments at this stage and the structural differences between the primary and permanent teeth, this study aimed to compare the microhardness of early enamel lesions of primary teeth treated with an infiltrant resin, a fluoride varnish and a product containing CPP-ACP.

2. MATERIALS AND METHODS

Sample preparation

The tooth samples were collected from private pediatric dentistry offices, within two months, and kept in normal saline solution at room temperature, for no more than two months, until the initiation of the study. According to Murakami et al. and Kumar et al. with 95% confidence interval and 95% test power and expert opinion, thirty six primary canines, devoid of caries, hypoplasia, restoration, cracks, and fractures, exfoliated or extracted for orthodontic purposes were included in the study using convenient sampling method. The study protocol was approved by the ethics committee of Jondishapur University of Medical Sciences (IR.AJUMS.REC.1398.574). The number of samples for each of the three study groups was calculated to be 12, with the total of 36 teeth. The teeth were immersed in a solution of thymol 0.1% (Aldrich, USA) for 48 hours to get disinfected. The debris and the remaining soft tissue were removed from the teeth by a sterile gas and a scalpel No. 12 (Juya, Iran). The teeth surfaces were cleaned thoroughly using a brush in a low-speed hand piece and a water spray. The teeth were stored in normal saline at the room temperature until the study began (Ekstrand et al., 2010). The roots of the teeth were removed at the cemento enamel junction (CEJ) using a diamond fissure bur (Tizkavan, Iran) in a high-speed handpiece. The crowns were embedded in a cube of self-cure epoxy resin (1cm×1cm×1cm) (Acropars 2000, Iran), with the buccal surfaces exposed. A total of 7 grams of epoxy adhesive was used for each mounting. Epoxy adhesive is highly stable and resistant to acidic solutions. The mounting method was such that the buccal surface of the crown was parallel to the cube cross-section. Leaving a 2 × 2 mm window at the center of the buccal surface, the entire buccal surface of the samples was covered by 2 layers of an acid-resistant varnish (Maxfactor-France) (Memarpour et al., 2015).

Creating artificial caries

The aim of this step is to demineralise the samples to simulate early carious lesions in the enamel blocks. The demineralizing solution was prepared in the laboratory of the Department of Chemistry, School of Pharmacy of Ahvaz Jondishapur University of Medical Sciences. The demineralizing solution contains 0.05 mM acetic acid, 2.2 mM CaCl₂, and 2.2 mM KH₂PO₄ with a pH of 4.4 (adjusted with 1M KOH). The samples were immersed in the demineralizing solution in an incubator (Innova-USA) at 37 °C for 4 days (96 hours). The pH of the solution was monitored daily using a pH metre (Metrohm, Swiss). After the allotted time, the samples were removed and rinsed with distilled water and air-dried gently (Eshghi et al., 2014; Kumar et al., 2008).

Microhardness Test

Vickers hardness testing machine was used in this in-vitro study to collect data about microhardness. The primary or secondary microhardness was measured by the Vickers testing machine (Innovatest, Netherlands). The microhardness was measured at three points with a distance of 100 microns and a force of 50 grams for 10 seconds, and the mean was recorded with the Vickers unit VHN (Vickers hardness number) and reported as the primary microhardness coefficient before the remineralization process. After the remineralization or application of the infiltrate, the resulted microhardness values were regarded as the secondary microhardness coefficient (Torres et al., 2012).

Experimental groups and the remineralization process or resin infiltrate application

After measuring the initial microhardness, the samples were randomly divided into the following three groups. Each sample within each experimental group was randomly attributed a number from 1 to 12. The attributed number was carved at the bottom of the resin cube using a dental bur. For group 1 specimens, the ICON resin infiltrant was applied according to the manufacturer's instructions: First, 15% hydrochloric acid gel (ICON-etch, Hamburg, Germany) was applied to the enamel surface for 2 minutes. Then the surface of the sample was washed with running water for 30 seconds and dried with a gentle pressure of air without water and oil so that no specific moisture remained on the surface. In the next step, the samples were dehydrated with 99% ethanol (ICON-Dry, Suring, DMG, Hamburg, Germany) for 30 seconds and again air-dried gently without water and oil. Placed in a special syringe by the manufacturer, the infiltrant resin (Dry, Suring, DMG, Hamburg, Germany) was then applied on the enamel surface for 3 minutes, and

the excess resin was removed using a cotton roll. The infiltrant resin was light-cured (Demi, Kerr, USA) for 40 seconds. According to the manufacturer's recommendation, the infiltrant resin was put on the tooth surface again. After one minute, the excess infiltrate on the surface was removed with a cotton roll. The infiltrant resin was light-cured for 40 seconds (Torres et al., 2012).

On specimens of group 2, GC Tooth Mousse paste (GC America INC; U.S.A) was applied for 3 minutes daily at a thickness of 1 mm, for 7 days (Somani et al., 2014). In group 3, the samples were gently air-dried, and fluoride varnish (Centrix; U.S.A) was applied for 1 minute, 3 times a day over a 10-day period, using the applicator in the varnish pack (McDonald, 2011). At the end of each working day, when the remineralization cycle is finished, all the treated samples were immersed in artificial saliva made in the chemical laboratory of School of Pharmacy, Ahvaz Jondishapur University of Medical Sciences. The artificial saliva was made according to McKnight–Hanes and Whitford formulation and contains 2 grams per litre of Methyl-p-hydroxybenzoate and 10 grams per litre of Sodium Carboxymethyl Cellulose and 0.625 grams per litre of KCl and 0.059 grams per litre of $MgCl_2 \cdot 6H_2O$ and 0.166 grams per litre of $CaCl_2 \cdot 2H_2O$ and 0.804 g/l K_2HPO_4 and 0.326 g/l KH_2PO_4 with a pH of 6.75 (adjusted by KOH) (McKnight –Hanes & Whitford, 1992). After 10 days, the samples were washed with distilled water and stored in normal saline solution. They were sent to the laboratory after 10 days for the secondary microhardness to be measured in Vickers unit.

Statistical analysis

Data were imported to SPSS software v 22. The pairwise comparison of the mean of the primary and secondary microhardness coefficients of the three groups was conducted using paired T-test.

3. RESULTS

In the fluoride varnish group, the microhardness coefficient increased from 138.6 (demineralisation) to 178.3 (remineralisation). The change was 39.7 and statistically significant ($P = 0.0010$) (Table 1) (Figure 1). In the group treated with GC Tooth Mousse, the coefficient of microhardness increased from 133.3 to 155.4. The coefficient of microhardness in this group was increased 22.1 V. This improvement was statistically significant ($P = 0.04$) (table 1) (Figure 2). However, change in the microhardness coefficient of the Resin infiltrant group was not significant ($P=0.2$). Regardless of the insignificance, the microhardness coefficient increased from 139.7 to 152.4 (Table 1) (Figure 3). Figure 4 shows a comparison of the increase in the microhardness coefficient in the two groups treated with the fluoride varnish and GC Tooth Mousse. Although the improvement in the group treated with the fluoride varnish was higher, this difference was not statistically significant (Table 2).

Table 1 Evaluation of the microhardness coefficient in Vickers unit after the treatment with varnish fluoride, GC Tooth Mousse, resin infiltrant

Experimental groups		Mean±SD	Paired T-test results
Varnish Fluoride	Demineralization	138.6±48.38	t=4.416 df=11 P= 0.0010*
	Remineralization	178.3±57.81	
	Diff	39.75±31.18	
GC Tooth Mousse	Demineralization	133.3±39.21	t=2.261 df=10 P= 0.0473*
	Remineralization	155.4±46.68	
	Diff	22.1±32.54	
Resin Infiltrant	Demineralization	139.7±42.98	t=2.11 df=7 P= 0.072 ^{NS}
	Remineralization	152.5±38.05	
	Diff	12.8±17.19	

-Diff: the difference between the remineralization microhardness value compared to the demineralization microhardness value (Remineralization-Demineralization) *P values<0.05 ^{NS}not significant

Table 2 Comparison of the changes in microhardness coefficient in Vickers unit after the treatment with the fluoride varnish and GC Tooth Mousse

Variable	Varnish Fluoride	GC Tooth Mousse	Are the slopes equal?
Best-fit values			F = 0.372977. DFn=1 DFd=42 P=0.5447 ^{NS}
Slope	39.75 ± 21.76	22.18 ± 18.38	
Y-intercept when X=0.0	98.84 ± 34.41	111.1 ± 29.06	
X-intercept when Y=0.0	-2.487	-5.006	
1/slope	0.02516	0.04508	

^{NS} Not significant

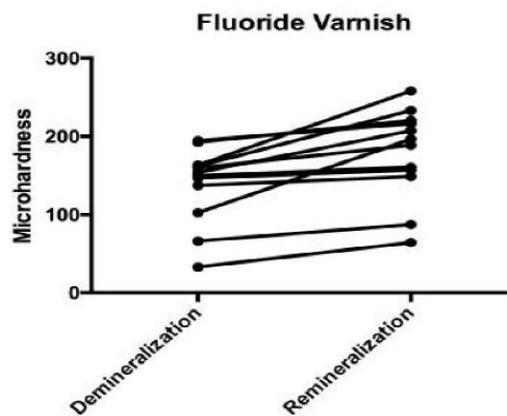


Figure 1 Evaluation of the changes in microhardness coefficient after the fluoride varnish treatment

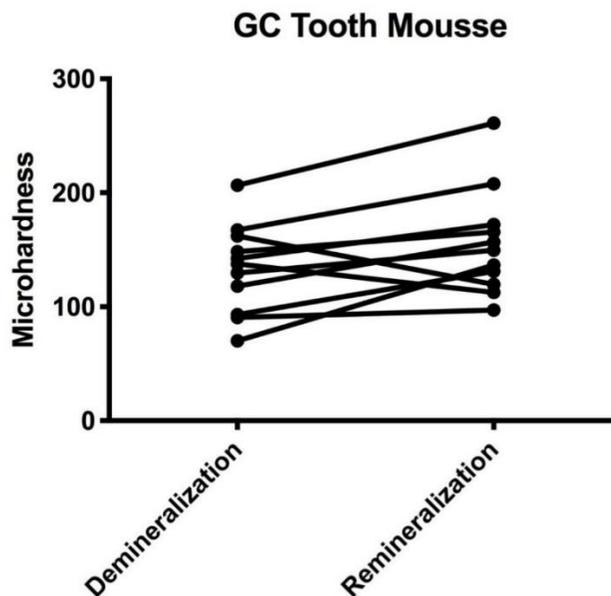


Figure 2 Evaluation of the changes in the microhardness coefficient after the treatment with GC Tooth Mousse

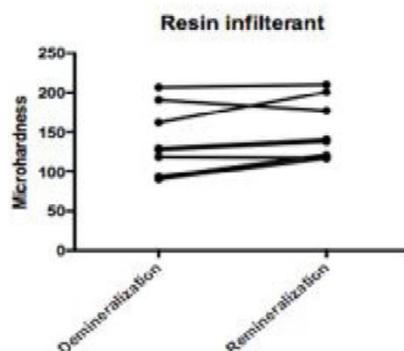


Figure 3 Evaluation of the changes in the microhardness coefficient after the treatment with the infiltrant resin

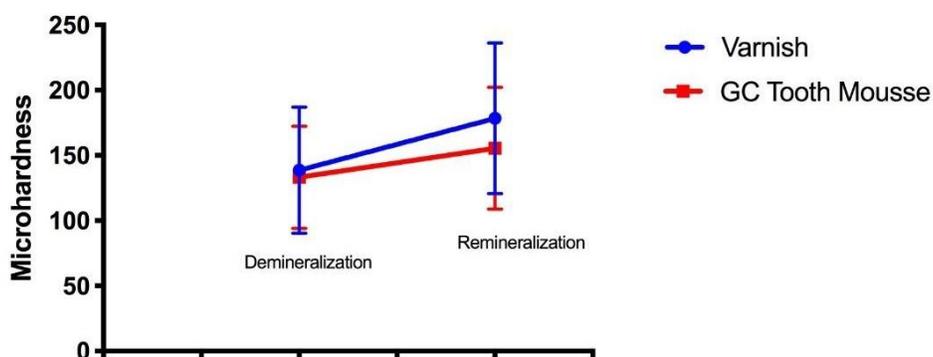


Figure 4 Comparison of the changes in the microhardness coefficient after the treatment with the fluoride varnish and GC Tooth Mousse

4. DISCUSSION

Today, the prevention of dental caries is more important than its treatment. One of the problems in pediatric dentistry is the development of rapid and progressive caries, in young children. Adolescents have increased risk of caries due to the hormonal changes, compatibility problems, disobedience and excessive consumption of carbohydrates, sweets and snacks (McDonald, 2011). One of the goals of preventive dentistry in children and adolescents is to prevent caries in early stages and remineralize the tooth surface; this will reduce the development of cavities and preserve more tooth structure (Zamarano et al., 2017). In general, two groups of treatments can be used to treat early caries: 1. materials affecting the mineralization of enamel by replacing lost ions from enamel prisms (Cochrane et al., 2010); 2. Substances, such as sealants and infiltrates, which reduce the absorption and penetration of carious substances such as acids, toxins and bacterial metabolites into the tooth, structure (Ericson et al., 2003). Infiltrant resin technique is a new and successful idea for treating early caries, without cavitation, extended to the outer one-third of the dentin. Besides, this method is a micro-invasive treatment, requiring no cavity preparation or local anesthesia. Subsequently, the procedure is not painful or annoying to the patient (Andrej et al., 2010). The added advantage of the infiltrant resin technique is that the resin fills the pores within the enamel of the early non-cavitated carious lesion. It also makes the tooth structure resistant against acidic attacks, preventing the caries progression (Behrouzi et al., 2019). ICON-etch have been found to be more effective in surface erosion of the lesions, compared to 37% Phosphoric acid. More etching time with ICON-etch induces higher resin depth of penetration. The use of ICON-Dry reduces the contact angle and viscosity leading to higher resin penetration. ICON-Infiltrate is a methacrylate-based resin, consisting of Bis-GMA and TEGDMA. Elevated concentration of HEMA, TEGDMA and ethanol causes greater resin penetration (Behrouzi et al., 2019). By this time, most studies have been conducted on permanent human teeth. In the present study, the effect of three different materials including an infiltrant resin, a fluoride varnish and GC Tooth Mousse on the microhardness of primary enamel lesions in-vitro was evaluated. Assessment of enamel microhardness coefficient was performed using Vickers testing machine. The advantage of this method is the high accuracy as well as the ability to measure quantitatively (RehderNeto et al., 2009). Due to the high prevalence of early carious lesions in primary dentition and the importance of using non-invasive or less invasive

techniques, the necessity for such a study existed. However, our results indicated that, despite a slight increase in the microhardness coefficient of the enamel lesion, resin infiltrate did not change the enamel microhardness significantly. In a clinical study, Kumar and co-workers investigated the effect of an infiltrant resin on the micromechanical properties of the hypomineralized teeth. They indicated that the penetration of resin infiltrate gave rise to higher, but insignificant ($P=0.56$) Knoop hardness number compared to the control group (Kumar et al., 2017). In contrary to this finding, Berek et al. and colleagues, investigating the effect of the remineralizing factors on the demineralized early enamel carious lesions of permanent teeth, showed that the resin infiltrate induced the best results in elevating the microhardness values compared to NaF and nanohydroxyapatite (Barek et al., 2018). Berek et al.'s method was similar to ours. The contrast in their results compared to ours can be attributed to the higher enamel thickness in the permanent teeth. Turska-Szybka and workmates demonstrated that sealing the non-cavitated lesions with the resin infiltrate in primary dentition, combined with the use of fluoride varnish, attenuate the probability of lesions progression by 92.8% (Turska-Szybka et al., 2016). Our findings also revealed that the fluoride varnish and GC Tooth Mousse significantly increased the microhardness coefficient. Although the improvement in the microhardness in the fluoride varnish-treated teeth was greater than GC Tooth Mousse, the difference was not statistically significant. Therefore, it can be stated that both substances have a similar effect on improving enamel remineralization. These findings were consistent with other studies. Nalbantgil and team mates showed that the microhardness was significantly higher than the control group after using the fluoride varnish (Nalbantgil et al., 2013). Priyadarshini and colleagues also conducted a comparison of enamel surface hardness after topical application of neutral sodium fluoride and organic fluoride in the form of amine fluoride. They declared that both substances help the remineralization process by reducing the demineralized enamel crystals (Priyadarshini et al., 2013). Dionysopoulos and co-workers evaluated the effects of fluoride treatment on bleached enamels and concluded that topical fluoride increased the surface microhardness of the enamel (Dionysopoulos et al., 2017). Tooth enamel can be considered as a low soluble salt in aqueous solution (saliva for instance). There is an ideal balance between demineralization and remineralization in the saliva. Fluoride promotes remineralization and speeds up the process with the calcium in the saliva (Bizhang et al., 2006). Therefore, the study tried to simulate clinical conditions and artificial saliva was used to store the teeth. Also, the storage conditions in the incubator were set at 37° C and every attempt were made to make the in-vitro condition similar to the mouth. However, clinical studies are needed to confirm these findings.

Despite the high ability of infiltrant resin to prevent the progression of early caries of primary and permanent dentition, the following points should be considered: 1) Hydrochloric acid and ethanol have an unpleasant odour, and can be annoying for the children, compromising their cooperation (Meyer-Lueckel & Paris, 2008). 2) The total duration of treatment with the infiltrant resin is relatively long. (2 minutes for etching the surface, 30 seconds for water irrigation, 30 seconds for applying the ethanol, 3 minutes to allow the resin to penetrate into the lesion, 40 seconds for light curing and according to the manufacturer's recommendation, 1 minute for reuse of the resin and 40 seconds for light curing). Considering that the time factor in pediatric dentistry is one of the most important factors (Andrej et al., 2010). 3) The cost of infiltrant resin technique is much higher than oral hygiene advices and the use of fluoride. Due to the shorter lifespan of deciduous teeth in the mouth compared to permanent teeth, many parents may demand for simpler, less expensive treatments for their child's primary teeth (Liu et al., 2013). Nevertheless, in 2013, Crombie and colleagues declared that resin infiltrate improved the clinical appearance of the teeth in patients with hypomineralized molars and incisors (Crombie et al., 2014). In another study by Munoz and team mates in 2013, resin infiltrate was useful in solving the tooth discoloration-related appearance problems caused by moderate to severe fluorosis (Muñoz et al., 2013). According to a study in 2013 by Liu and workmates, infiltrant resin has a greater penetration depth and can penetrate to the outer half of the dentin (Liu et al., 2013). As previously discussed, because of greater penetration depth and exerting a sealing effect on the lesion against the bacteria and their toxins, infiltrant resin had a greater effect on preventing the progression of early caries of primary dentition toward cavitated lesions and involvement of the pulp, compared to fluoride varnish and oral hygiene advices (Ericson et al., 2003; Turska-Szybka et al., 2016). Generally, the cost-benefit ratio of the resin infiltrate method is more appropriate and more effective in older children, with better cooperation, who are prone to early enamel lesions due to orthodontic treatments, hormonal and dietary (including fluoride consumption) changes.

5. CONCLUSION

All three substances studied increased enamel microhardness. Fluoride varnish had the highest recovery rate of micro-hardness, and to a lesser extent GC Tooth Mousse and then infiltrant resin increased enamel surface micro-hardness by improving the remineralization process. Infiltrant resin had a positive effect on improving the enamel microhardness coefficient, but this change was not statistically significant. Fluoride varnish had more improvement than GC Tooth Mousse, but this was not statistically significant.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data and materials availability

All data associated with this study are present in the paper.

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