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Original research

The effects of resin infiltration on demineralized root surface: An experimental study

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Purpose

The objective of this experimental invitro study was to investigate the effects of resin infiltration (RI) on surface roughness, microhardness, color and surface characteristics of artificially demineralized root surfaces.

Materials and Methods

Forty-two root specimens prepared from freshly extracted intact human upper incisors were subjected to surface roughness, microhardness, and color tests. Profilometer was used to measure surface roughness and Vicker's Hardness tester was used to measure the changes in microhardness. The color measurements were performed by a spectrophotometer using the CIELAB parameters. Following measurements, specimens were divided into 3 groups (n=14): G1: intact root surfaces, G2: demineralized root surfaces and G3: Resin infiltrated root surfaces (Icon, DMG) following demineralization. Surface roughness, microhardness and color measurements were repeated in G2 and G3 after demineralization and RI. One specimen from each group was examined by SEM. Data were analyzed statistically ($p<0.05$).

Results

Application of RI to artificially demineralized root surfaces significantly decreased the surface roughness ($p<0.001$) and increased the microhardness ($p=0.023$). RI also affected the color of demineralized root surfaces. SEM examinations revealed that the porosities on demineralized root surfaces seemed to be sealed after RI.

Conclusion

RI affected the surface roughness, microhardness, color and surface appearance of artificially demineralized root surfaces.

Keywords: *Resin infiltration, root surface caries, microhardness of root surface, roughness of root surface, color of root surface*

Introduction

Root caries is a global public health issue, causing the breakdown of remaining natural and restored teeth, especially in elderly patients (1, 2). Gingival recession because of periodontal disease, xerostomia as well as traumatic tooth-brushing habits increase the susceptibility of root caries as the thin cementum can be easily removed. In addition, due to reduced salivary flow in elderly patients, biofilm and plaque accumulation increase easily and also act as risk factors for root caries formation (3). Moreover, due to the lower mineral content of cementum, demineralization rapidly occurs on even at higher pH values compared to enamel and dentin (4).

In the management of root caries lesions, the main treatment principle is firstly to prevent the caries formation by reducing the caries risk factors and diagnose the caries lesions in the early phase when formed, thereby, reducing the need for more invasive treatments. In this regard, several approaches have been beneficial such as preventive measures, mainly reha-

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bilitation of oral hygiene, as well as the topical application of remineralizative antimicrobial agents and resin sealers (5, 6). However, in case restorative treatment is required, the least invasive methods should be chosen (7).

Low-viscosity resin infiltration (RI) into the porosities of initial caries lesion is a relatively new treatment modality for the early enamel caries lesions (8, 9). In this technique, resin monomers can penetrate into the initial lesions up to 34–58 µm in depth, after hypermineralized enamel is removed with 15% hydrochloric acid for 2 min. The unique caries-inhibiting effect of RI is based on the occlusion of the spaces within the body of the lesion. RI only requires one visit and mechanically stabilize the fragile lesion structure (10-13).

It has been shown that the coating of root surfaces with a resin-based material, which has the capacity of release and recharge the fluoride ions was effective in the protection of the root surfaces against demineralization (6).

Various experimental and in vivo studies have reported that RI inhibits the progression of early carious lesions of enamel and increasingly recommended it for clinical use (11-14). However, the effect of RI on initial root surface lesions has not been investigated. Therefore, the purpose of this in vitro experimental study was to examine the effects of RI on artificially demineralized root surface lesions in terms of surface roughness, microhardness, and color. The null hypotheses were that; RI application would not change neither of the surface roughness, microhardness nor the color of artificially demineralized root surfaces

Material and Methods

Ethical statement

The study protocol was approved by the non-interventional clinical researches ethics boards of the university.

Sample size determination

The power of the sample size was calculated by a flexible statistical power analysis program for the social, behavioral, and biomedical sciences (G*Power, version 3.1, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) with a 95% confidence interval, and 80% power, and 0.50 effect size values for n=42 (14 in each group) according to one-way ANOVA-type power analysis (15, 16).

Specimen preparation

Forty-two intact human permanent maxillary incisors extracted due to the periodontal reasons in the last 6 months were used in the study. Teeth having roots without any other defects were included after cleaning from soft tissues using slurry of pumice and stored in 0.1% thymol solution to avoid bacterial growth. Root surfaces of the teeth were examined by a stereomicroscope (American Optical, Buffalo, NY, USA) at ×40 magnification and then the roots were cut 1 mm below from the cemento-enamel junction using a diamond saw at low speed (Isomet 2000, Buehler Ltd., Lake Bluff, IL, USA) under water coolant and embedded in acrylic resin (Vertex, Vertex-Dental B.V, Soesterberg, Netherlands) facing the buccal surfaces upward. The root specimens were ground flattened using 2400-, and 4000- grid silicon carbide papers to obtain standardized and well-polished surfaces. The root surfaces were re-examined under the stereomicroscope to ensure cementum was not removed. After the specimens were ultrasonically cleaned in distilled water for 2 min, they were partly coated with a nail varnish resistant to acidic challenge, leaving a window of 4 × 4 mm on the cementum.

The polished root specimens (n=42) were subjected to surface roughness, microhardness and color tests. Surface roughness was measured by a contact type profilometer (Perthometer M2, Mahr, Gottingen, Germany), microhardness was measured by Vicker's hardness tester (Microhardness Tester, Shimadzu, Kyoto, Japan), and color readings were obtained by a spectrophotometer (VITA Easyshade, Advance, Zahnfabrik, Bad Säckingen, Germany).

Surface Roughness (Ra, mm) measurement

Surface roughness was detected via a contact- type profilometer with a stylus. After placing the specimens on a flat surface, the needle of the device was put on the root specimen surface, and recordings were done. Five Ra measurements were taken from each specimen within the measuring length and an arithmetic mean value was calculated. After each 5 specimens, the device was calibrated to ensure reliable readings.

Surface microhardness (VHN) measurement

Microhardness of each specimen was measured with a microhardness tester using a 980 g load with a 20 s dwell time. VHN was calculated by making five indentations on the center of each specimen and 0.5 mm apart from each other. The arithmetic mean value was calculated from each specimen for the statistical analysis. After measurement of each specimen, the device was calibrated.

Color measurement

Color of each specimen was measured by a dental spectrophotometer against a white background. Shade of each specimen was detected based on L*, a*, and b* values, in which L* is the value of 0 (black) to 100 (white), a* is the amount of red and green, and b* is the amount of yellow and blue. Color change was calculated via the following formula (17):

$$\Delta E=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$$

Calibration of the device was performed after 5 readings by the ceramic reflectance standard calibration block of the device.

Study groups

After surface roughness, microhardness and color measurements, the specimens were randomly allocated into 3 groups: G1 (n=14) (Negative control): Intact root surfaces were stored in artificial saliva throughout the study which was replaced with fresh artificial saliva every day. Artificial saliva was prepared according to the formula described by of Gohring *et al.*(18). The pH was arranged 7.4 - 7.8 throughout the experimental period to avoid dehydration. G2 (n = 14) (Positive control with demineralised root surfaces): Demineralization of root surfaces were generated according to the protocol of Ten Cate and Duijsters (19, 20). The entire specimen bodies were immersed in a buffer solution in disposable container containing demineralizing solution for 72 hr at room temperature (23+1 °C). The pH was monitored daily. After 72 hours, the specimens were cleaned in distilled water approximately for 1 min and dried with mild-air for 5 min. The demineralized root surfaces were evaluated under the stereomicroscope. G3 (n = 14): In this group, RI (Icon Resin Infiltration; DMG, Hamburg, Germany) was applied to the root surfaces following demineralization using the same protocol in G2. The manufacturer's instructions were strictly followed to perform RI. First, the dried root surface was etched with Icon-etch (hydrochloric acid) for 120s, and then rinsed for 30 s. After, Icon-dry was used for 30 s for dehydration, Icon-Resin infiltrant was gently applied on the specimen with an applicator tip and left undisturbed for 3 min. Then a gentle air was blown, and light-irradiated for 40 s by LED curing unite (Translux Power Blue, a wavelength of 460–470 nm and a light output of 600–1,550 mW/cm^2, Kulzer GmbH, Hanau, Germany). Icon-Resin infiltrant was applied again for an extra 1 min and light-irradiated for 40 s. Finally, 4000- grit, aluminum oxide impregnated abrasive paper was used for polishing of the specimen surface.

The surface roughness, microhardness and color measurements were repeated for G2 and G3 specimens.

Scanning electronic microscopy (SEM) analysis

One representative specimen from each group was mounted on aluminum stubs, sputter-coated and submitted to SEM analysis (JSM-6400, JEOL, Tokyo, Japan). Images were obtained at ×50, ×100, and ×200 magnifications.

Statistical analysis

Normality was evaluated by Shapiro Wilk test. Test of homogeneity of variances was performed by Levene's test. Intragroup comparisons were performed by one-way ANOVA, followed by Tukey HSD multiple comparisons test. When the assumption of homogeneity of the variance was not met, Welch test as an alternative to one-way ANOVA and Games-Howell test as an alternative to Tukey's HSD test was conducted. One-way repeated-measures ANOVA was used to compare the color changes of the groups followed by Sidak's multiple comparison test. P<0.05 was considered as significant.

Results

Table 1 presents mean, median, SD and min-max Ra of the groups. Shapiro Wilk test revealed that the data was normally distributed and Levene's test indicated non-homogeneity of variances p<0.05). Significant differences were revealed among the groups (p<0.001) by Welch test. The highest Ra was observed in G2, which the specimens were demineralized, while the lowest Ra was observed in G1. Welch test showed that, application of RI significantly decreased the Ra of demineralized root surfaces (p<0.001) but did not reach to the Ra of intact root surfaces (Table 1).

Table 2 presents mean, median, SD and min-max VHN of the groups. Shapiro-Wilk test showed that the data was normally distributed and Levene's test concluded the homogeneity of variances. One-way ANOVA test showed that there were also significant differences among the groups (p<0.001). Highest VHN was observed in G1, while the lowest was observed in G2. RI increased the VHN values but did not reach to the VHN of intact root surface (Table 2).

Shapiro Wilk test showed the data was normally distributed and Mauchly's Test of Sphericity indicated that the assumption of sphericity was met (p>0.05). The highest ΔE was calculated after demineralization of intact root surfaces. According to the one-way repeated measures ANOVA, there were statistically significant differences among the ΔE of the three groups (p=0.029).

The SEM images of intact, demineralized and RI applied root surfaces are demonstrated in Figure 1, Figure 2 and Figure 3. Photomicrographs of representative groups were taken at x50, x100 and x200 magnifications. Figure 1a,1b and 1c illustrate the intact root surfaces. Micrographs seemed to have smooth root surfaces. No morphological changes were detected at different magnifications. Only some scratches were seen on the surfaces. Figure 2a, 2b and 2c illustrate demineralized root surfaces at x50, x100 and x200 magnifications. Demineralized root surfaces with irregular appearances were detected. Figure 3a, 3b and 3c illustrate RI applied root surfaces. At different magnifications, it was seen that RI application to the demineralized root surfaces seemed to seal the root surface porosities resulting the surfaces appeared smoother. Table 3 presents color change (ΔE) of the groups.

Table 1: *Mean, SD, median, and min-max of surface roughness (Ra, μm) of the groups.*

	Mean (SD)	Median	Min - Max	p*
Intact root surface	0.192 (0.049) [a]	0.182	[0.141 – 0.324]	<0.001
Demineralized root surface	0.67 (0.056) [b]	0.674	[0.569 – 0.773]	
Resin infiltrated root surface	0.322 (0.085) [c]	0.320	[0.201 – 0.447]	

* Welch test (p<0.05). Different letters denote statistically significant differences

Table 2: *Mean, SD, median, and min-max of surface microhardness (VHN) of the groups.*

	Mean (SD)	Median	Min - Max	p*
Intact root surface	65.22 (5.25) [a]	65.80	[56.70 – 73.80]	<0.001
Demineralized root surface	37.87 (4.14) [b]	37.74	[28.39 – 43.64]	
Resin infiltrated root surface	54.91 (3.72) [c]	55.15	[49.30 –59.64]	

* One-way ANOVA test (p<0.05). Different letters denote statistically significant differences

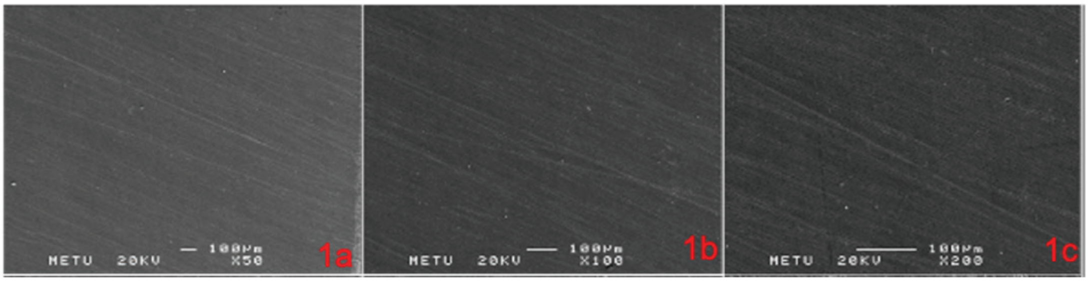

Figure 1. *SEM images of intact root surfaces.*
×50 (1a), ×100 (1b), ×200 (1c) magnifications

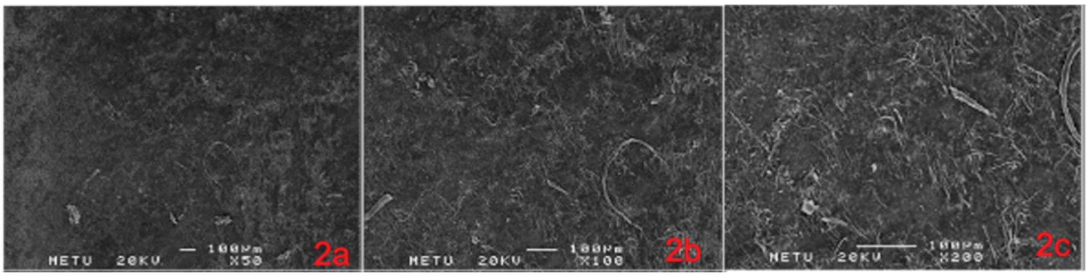

Figure 2. *SEM images of demineralized root surfaces.*
×50 (1a), ×100 (1b), ×200 (1c) magnifications

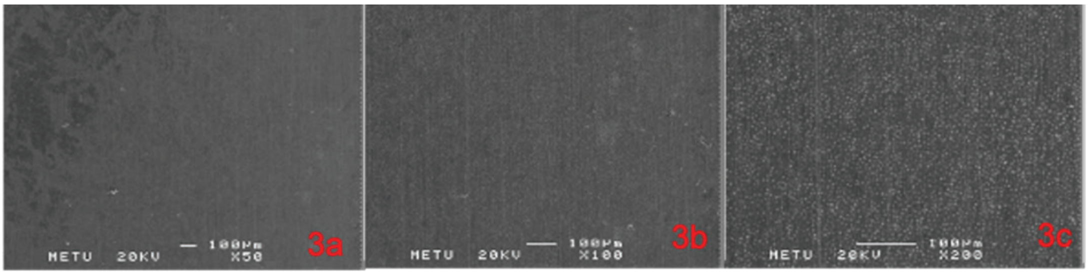

Figure 3. *SEM images of resin infiltrated root surfaces.*
×50 (1a), ×100 (1b), ×200 (1c) magnifications

Table 3: *ΔL, Δa , Δb, and calculated ΔE of the groups.*

Pairs	ΔL Mean (SD)	Δa Mean (SD)	Δb Mean (SD)	ΔE Mean (SD)	p*
Intact root surface - Demineralized root surface	9.46 (7.64)	-1.79 (1.40)	-8.33 (5.55)	14.11 (7.01) [a]	
Demineralized root surface - Resin infiltrated root surface	7.91 (7.82)	0.47 (1.31)	-3.96 (7.38)	12.61 (5.56) [b]	0.029
Intact root surface - Resin infiltrated root surface	-1.55 (7.86)	1.48 (1.35)	4.37 (6.08)	9.58 (5.25) [c]	

*One-way repeated measures ANOVA test ($p<0.05$). Different letters denote statistically significant differences

Discussion

Considering the prolonged lifetime in today's conditions, as the proportion of dentate elderly population is getting higher, the prevalence of root caries has been also increased. So, preventive and arrestive applications on root caries is crucial, especially to sustain the functional occlusion for better quality of life of elderly.

To inhibit lesion progression initiated on root surface, coating of the lesion surface with an adhesive material was recommended in several studies (21-23). Although promising results showing reduced severity of root surface lesions after coating, coating with adhesives was unable to fully resist the demineralization induced by acid attack (6). One reason for this phenomenon may be that the coating thickness of the adhesives was too thin to act as a substantive physical barrier against demineralization (22).

Recently, a resinous coating material with pre-reacted glass-ionomer fillers was investigated for the prevention of root caries. Ma *et al.* (6) examined the ability of this coating for protecting the root surface from further demineralization and reported that this pre-reacted glass-ionomer coating resin could be an effective material for protecting root surface from both chemical and biological challenges.

Resin infiltration (RI) was developed for the management of smooth surface and proximal non-cavitated caries lesions in which the porosities of enamel lesion are infiltrated with a low viscosity resin, creating a diffusion barrier within the lesion without establishing any material on the enamel surface delaying the time for restoration placement (13, 14, 24, 25). However, to the extent of the authors' knowledge, up to date only one study focused on the use of this technique on initial root surface lesions (26). In this study, Zhou *et al.* (26) evaluated the penetration of RI on root caries induced by Streptococcus mutans biofilms by fluorescent microscope, swept-source optical coherence tomography and confocal laser scanning microscope. They reported that RI had a good penetration ability and preventive effect on root caries. However, an additional risk factor of cervical enamel loss was identified.

In the present experimental in vitro study, the effect of RI on the surface roughness, microhardness and color of artificially demineralized root surfaces were investigated. As there have been no other studies conducted on this topic, the results of the present study were discussed with the effects of RI on early enamel demineralization.

Physicochemical properties of a material and tooth surfaces including surface roughness carries great importance since rough surfaces accommodate retention areas for both in vitro and in situ biofilm formation and so caries development (8).

It has been previously stated that the surface of RI applied enamel lesions may remain irregular even after finishing and polishing procedures (27). Inadequate RI to demineralized surfaces, the polymerization shrinkage and / or the interference of ethanol in the polymerization can play an important role (28, 29). Another factor may be the hydrochloric acid used for etching protocol. It was reported that, etching with 15% hydrochloric acid produced grooves and cracks in enamel (30). Considering the higher organic content and vulnerable structure of root surface to the acidic challenges, it was thought that hydrochloric acid, as a strong acidic agent, increased the surface roughness of root surfaces in the present study compared to intact root surfaces. However, application of RI to demineralized root surfaces resulted in significantly lower surface roughness values. This may probably be due to the resin, infiltrated into the demineralized root surfaces. Therefore, the first null hypothesis was rejected. Under clinical conditions, the surface roughness may show variations after etching compared to the obtained in vitro results considering the dynamic nature of the oral cavity. Thus, this aspect requires further research before making any clinical recommendations on root surface etching procedures.

The microhardness of resin infiltrated surfaces has been reported to be affected by the demineralization level of the surface, RI depth and the chemical composition of infiltrating resin agent (31, 32). The used resin monomers, such as TEGDMA-based resins, could increase the elasticity of the surface (33). For this reason, the results should be cautiously interpreted since, after indentation, elastic deformation may interfere the results, as previously shown in the comparison of micro- and nano- indentation procedures (34). In the present study, RI treated root surface lesions showed significantly higher microhardness values than the deminerized root surfaces. So, the second null hypothesis, regarding RI application would not change the microhardness of root surface lesions was rejected. This shows the penetration ability of the low-viscosity resin with high penetration coefficient for filling the pores within the remained crystal structures of the lesion and rehardening facility of the demineralized tissue. This result was also conformed with the SEM images in the current study. The images showed that, porosities on demineralized root surfaces seemed to be mostly sealed.

RI technique has been shown to mask white spot lesions of enamel (14, 35, 36). In this experimental study, the application of RI to the root surfaces resulted in a significant color change. Therefore, it has also a positive effect in masking the demineralization of root surfaces. This may be due to the fact that micro porosities of resin infiltrated demineralized root surfaces were filled with resin infiltrant instead of water and/or air. So, the third null hypothesis was also rejected.

The results of this study differ from the in vitro studies that have been performed already on enamel. It is important that the pretreatment with 15% hydrochloric acid has the potential of damaging susceptible cervical parts of the vital teeth structures. So, it should not be ignored. However, when this strong acid contacts soft tissues it has been shown to be harmless (37).

According to results of the current study, resin infiltration can be considered as an effective application option for the restoration of artificially demineralized root surfaces owing to its penetration ability, provision of better surface characteristics including microhardness and surface roughness, and recovering of color. Thus, Resin Infiltration is an effective microinvasive technique that seem better suited to treat artificially demineralized root surfaces.

Conclusion

Within the limitations of this experimental in-vitro study, it can be concluded that resin infiltration of the artificially demineralized root surfaces decreased the surface roughness and increased the micro hardness, additionally affected the color stability.

Türkçe öz: *Rezin infiltrasyonunun demineralize kök yüzeyi üzerine etkileri: Deneysel bir çalışma Amaç: Bu deneysel invitro çalışmanın amacı, rezin infiltrasyonunun (RI) yapay olarak demineralize edilmiş kök yüzeylerinin yüzey pürüzlülüğü, mikrosertliği, rengi ve yüzey özellikleri üzerindeki etkilerini araştırmaktır. Gereç ve yöntem: Yeni çekilmiş sağlam insan üst keser dişlerinden hazırlanan kırk iki kök örneği, yüzey pürüzlülüğü, mikrosertlik ve renk testlerine tabi tutuldu. Yüzey pürüzlülüğünü ölçmek için profilometre ve mikrosertlikteki değişiklikleri ölçmek için Vicker's Hardness test cihazı kullanıldı. Renk ölçümleri, CIELAB parametreleri kullanılarak bir spektrofotometre ile gerçekleştirildi. Ölçümlerin ardından örnekler 3 gruba ayrıldı (n=14): G1: sağlam kök yüzeyleri, G2: demineralize kök yüzeyleri ve G3: Rezin infiltre edilmiş kök yüzeyleri (Icon, DMG). Demineralizasyon ve RI sonrası G2 ve G3 örneklerinin yüzey pürüzlülüğü, mikrosertlik ve renk ölçümleri tekrarlandı. Her gruptan birer örneğin yüzeyleri SEM ile incelendi. Veriler istatistiksel olarak analiz edildi (p<0.05). Bulgular: Yapay olarak demineralize edilmiş kök yüzeylerine RI uygulaması, yüzey pürüzlülüğünü önemli ölçüde azalttı (p<0,001) ve mikrosertliği artırdı (p=0,023). RI, demineralize kök yüzeylerinin rengini de etkiledi. SEM incelemelerinde, demineralize kök yüzeylerindeki pörözitelerin RI'den sonra örtülendiği görüldü. Sonuç: RI, yapay olarak demineralize edilmiş kök yüzeylerinin yüzey pürüzlülüğünü, mikrosertliğini, rengini ve yüzey görünümünü etkiledi. Anahtar kelimeler: Rezin infiltrasyon; kök yüzeyi çürükleri; kök yüzeyinin mikrosertliği; kök yüzeyinin pürüzlülüğü; kök yüzeyinin rengi.*

Ethics Committee Approval: The study protocol was approved by the non-interventional clinical researches ethics boards of the university.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: UKV, AEY, ZB, SG participated in designing the study. UKV, AEY, ZB, SG participated in generating the data for the study. UKV, AEY, ZB, SG participated in gathering the data for the study. UKV, AEY, ZB, SG participated in the analysis of the data. UKV, AEY, ZB, SG wrote the majority of the original draft of the paper. UKV, AEY, ZB, SG participated in writing the paper. UKV, AEY, ZB, SG have had access to all of the raw data of the study. UKV, AEY, ZB, SG have reviewed the pertinent raw data on which the results and conclusions of this study are based. UKV, AEY, ZB, SG have approved the final version of this paper. UKV, AEY, ZB, SG guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared that they have no conflict on interest.

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