Original Article

Effect of bleaching with 15% carbamide peroxide on color stability of microhybrid, nanohybrid, and nanofilled resin composites, each in 3 staining solutions (coffee, cola, red grape juice): A 3-phase study

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ABSTRACT

Background: The literature on the effect of bleaching on stainability and color stability of any composites is scarce and controversial. In the case of some composites and/or bleaching agents or staining solutions, there is no previous study. Therefore, this rather large study was conducted for the first time to examine simultaneously the effects of bleaching and 3 staining solutions on 3 composite types. **Materials and Methods:** This 3-phase experimental *in vitro* study was performed on 18 groups of 5 specimens each: 90 composite discs with 10 mm diameter and 1 mm thickness were fabricated from 3 resin composites (microhybrid, nanohybrid, and nanofilled). Forty-five discs underwent bleaching with 15% carbamide peroxide, and the remaining 45 were immersed in distilled water at room temperature. The color assessment was performed before and after this step, and Δa , Δb , and ΔL color parameters were calculated for each specimen. The specimens were then immersed in coffee, cola, and red grape juice for 2 weeks, and underwent colorimetry again. The simultaneous effects of bleaching and staining agents and composite types on ΔE (color changes) of composite specimens were analyzed using an independent-samples *t*-test, a Mann–Whitney *U*-test, and two-way and one-way analysis of variances followed by a Tukey and a Dunnett's T3 tests ($\alpha = 0.05$).

Results: Bleaching had no significant effect on color changes of microhybrid, nanohybrid, or nanofilled composites (P > 0.05). The stainability of composites did not increase after bleaching (P > 0.05). Regardless of the composite type and the presence of bleaching, coffee had the worst effect on color of composite specimens, while cola had the smallest staining effect (P < 0.05).

Conclusion: Stainability of microhybrid/nanohybrid/nanofilled composites did not increase after bleaching with 15% carbamide peroxide. Coffee should be consumed with care, as far as composite coloring is a concern, regardless of the type of composite in use or its bleaching history. Cola was the weakest stainer. Coffee always caused perceptible staining ($\Delta E > 3.7$), regardless of the composite in use or its bleaching history. Red grape juice caused such perceptible colorations in most cases. Cola did not cause any perceptible discoloration in most cases.

Key Words: Colorimetry, composite resins, dental materials, discoloration, tooth bleaching

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INTRODUCTION

Considering the increasing demand of patients for cosmetic dental procedures, tooth-colored restorative materials are currently more commonly requested by patients. Despite the advancements in the quality of tooth-colored restorative materials in the recent years, the manufacturers still attempt to develop materials with improved durability, maximum esthetics, and minimum need for replacement.^[1-4] Discoloration is among the main reasons for the replacement of tooth-colored restorations after a period of clinical service in the oral cavity.^[1,5,6] Intrinsic and extrinsic factors are involved in the discoloration of resin restorations:^[1,2,5-10] Changes in the structure of resin or filler particles, and incomplete polymerization are among the intrinsic causes of discoloration of composite resin restorations. Absorption of external stains and pigments such as tea, coffee, and nicotine are among the extrinsic factors responsible for discoloration of composite restorations. Surface properties such as surface roughness can also affect the color stability and stainability of restorations. Size and type of filler particles, resin matrix composition, finishing and polishing technique, and bleaching treatments are among the influential factors in the surface roughness of composite restorations.^[1,2,5-10]

Bleaching has become a routine procedure of treating discolored teeth in dental offices. Since it is not possible to prevent the exposure of restorations to bleaching agent in different bleaching techniques, its effects should be examined. Office-bleaching using higher concentrations of hydrogen peroxide, and home-bleaching using lower concentrations of carbamide peroxide are among the safe cosmetic dental procedures.^[11,12] However, bleaching may negatively affect the color, surface smoothness, and microhardness of composite restorations as well as their further stainability.^[13-17]

Therefore, evaluating the effects of different bleaching protocols on color stability of various composite restoration materials under the influence of different staining solutions is necessary. In this regard, the literature has been actively examining various combinations of bleaching protocols, composite resins, and staining solutions. However, the studies are a few and controversial. Bleaching can make resin composites more prone to staining as a result of changing their surface properties and increasing micropores^[13-20] or even inducing microcracks;^[21] and this effect can differ depending on the duration of bleaching and the bleaching technique in use^[22-24] as well as the staining agent.^[25-27] For instance, 15% hydrogen peroxide and warm 30% hydrogen peroxide may discolor resin composites.^[28] Li et al.,^[29] observed discolorations in packable nanohybrid composite following bleaching with 15% carbamide peroxide. Hubbezoglu et al.[30] reported discoloration of resin materials as a result of exposure to concentrated hydrogen peroxide.^[30] On the other hand, Savic-Stankovic et al. in 2021^[2] asserted that bleaching might not affect the gloss and surface roughness of the nanohybrid and microhybrid resin composites.^[2] Telang et al.^[31] evaluated the influences of staining and ensuing bleaching using carbamide peroxide gel 15% on the color stability of three resin composites (supranano, nanohybrid, and silorane) stained using coffee or turmeric.^[31] They observed that nanohybrid composite had the greatest discoloration while supranano composite had the smallest extent of discoloration. Bleaching might reverse the staining effect in all the groups; this would be the greatest in nanohybrid composite. The supra-nano-filled composite maintained color stability and minimal surface roughness.[31] Rodrigues et al.[32] examined alterations in staining susceptibility of two resin composites (Filtek Z250 XT and Filtek Z350 XT) after bleaching using 35% hydrogen peroxide, 16% carbamide peroxide, or deionized water as a control group. According to them, bleaching caused minimal discoloration ($\Delta E^* < 1$) in all groups; Filtek Z350 XT composite was more prone to discoloration. They concluded that bleaching agents might not significantly discolor those 2 resin composites.^[32] Mohammadi et al.[33] assessed the effect of bleaching with 15% carbamide peroxide gel on color stability and surface topography of a microfilled resin composite and a giomer. They concluded that although bleaching increases the surface roughness of the giomer and microfilled composite, it might not cause clinically perceptible color alterations in either of them.^[33] Nevertheless, they did not subject their specimens to any staining agents.^[33] Of course, the staining solution may matter itself. For example, Bagheri et al.^[25] found that coffee could be the worst discoloring agent. On the other hand, Yazici et al.[26] reported no significant difference between discoloration potentials of coffee and tea, while Celik et al.^[27] even showed a greater discoloration influence of tea compared to coffee. Furthermore, turmeric causes greater discoloration compared to coffee.^[31]

Besides the scarcity and controversial nature of the literature, most previous studies are limited to a narrow research scenario, such as the effect of bleaching on discoloration of one or two composite types. Thus, many aspects of this dynamism remain unstudied or under-studied, as indicated by a 2022 systematic review.^[1] Hence, this rather large 3-phase study aimed to assess simultaneously, for the first time, the effects of 3 staining agents (coffee, cola, and red grape juice) as well as bleaching on color parameters of 3 different composite resin types (and their interactions). The null hypotheses were that different staining solutions would have the same effect on composite resins' color; and that the bleaching treatment would have no significant effect on color stability and stainability of each of these 3 composite resins.

MATERIALS AND METHODS

This experimental 3-phase study was conducted as 3 consecutive DDS (Doctor of Dental Surgery) theses as 3 phases of one research. Three different composites investigated: A microhybrid were composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA), a nanohybrid composite resin (Filtek Z-350XT, 3M-ESPE), and a nanofilled composite (Filtek Z-250XT, 3M-ESPE). Moreover, a bleaching agent 15% carbamide peroxide (Opalescence, Ultradent, St. Jordan, UT, USA) was used in this study. Table 1 presents the chemical composition of the used materials. Since the study was in vitro, no harm was done to any humans or living beings, and thus the study ethics and protocols were approved for each of the 3 subsequent theses, by the Research Committee of the University. No ethics code was issued.

Sample size

For each of the 6 major groups (all staining solutions combined within each composite-bleaching group), the sample size was determined as 15 specimens per group. A proper number for color assessment studies is usually adopting 3–5 specimens per sub-group, due to the high precision of colorimetry. We chose the upper limit (i.e., 5 specimens). There were 18 subgroups; this amounted to 90 specimens overall in all the 18 subgroups. For this rather large sample size, 3 research projects (DDS theses) were defined and conducted in 3 phases.

Preparation of specimens

A total of 90 disc-shaped composite specimens 10 mm in diameter and 1 mm thin were fabricated using a tetrafluoroethylene mold covered with a clear polyester strip (Mylar strip; Henry Schein, Melville, NY, USA). Composite resin specimens were then light-cured from the top surface using Valo light-curing unit (Ultradent Product, South Jordan, UT, USA) at a light intensity of 1000 mW/cm² in standard mode for 20 s. The light output power was measured and calibrated using a radiometer (Kerr, Demetron, Orange, CA, USA) after every 10 times of irradiation. To obtain a standard smooth surface, the top surfaces of the specimens were polished with a 1200-grit silicon carbide abrasive paper under water spray. Next, the specimens were stored at room temperature for 2 weeks.

Colorimetry 1: Before bleaching

The baseline L*, a*, and b * color parameters were measured three times for each specimen according to CIE L * a*b* color space using a spectrophotometer (Ihara SpectroCAM Scanning

Material	Composition	Manufacturer		
Microhybrid composite	Filler: 0.0–3.5 µm, 83% wt	3M-ESPE		
Filtek Z250 Shade A2	Matrix: Bis-EMA; UDMA	ST Paul, MN, USA		
Nanohybrid composite	Filler: 20–50 nm, 78.5% wt	3M-ESPE		
Filtek Z350XT Shade A2	Matrix: Bis-EMA, UDMA, TEGDMA Bis-EMA	ST Paul, MN, USA		
Nanofilled composite	Filler: ≤3 µm, 20 nm; 82% wt	3M-ESPE		
Z250XT Shade A2	Matrix: Bis-GMA, Bis-EMA, DEGDMA, TEGDMA	ST Paul, MN, USA		
Bleaching agent:	15% carbamide peroxide	Ultradent, South Jordan,		
Opalescence 15%	3% potassium nitrate	UT, USA		
carbamide peroxide	C 2% fluoride carbopol			
	glycerin			
	flavoring agents			

Table 1: Chemical composition of the main materials used in this study

Bis-GMA: Bisphenol A-glycidyl methacrylate; UDMA: Urethane dimethacrylate; Bis-EMA: Bisphenol A ethoxylated dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; DEGDMA: Diethylene glycol dimethacrylate

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spectrophotometer, Ihara, Aichi, Japan), and the mean of the three values for each specimen was calculated and recorded. The spectrophotometer was calibrated with a standard white cardboard before measurements of each specimen. The L* variable is an indicator of brightness with 0 meaning absolutely dark and 100 meaning absolutely bright. The a* parameter is a chromatic coordinate for the green-red spectrum with positive values meaning shades of red and negative values indicating shades of green. The b* parameter is another chromatic coordinate for the blue-yellow spectrum with positive values showing shades of yellow and negative values indicating shades of blue.

Bleaching treatment

The specimens were randomly divided into two "bleaching" groups (i.e., bleached versus nonbleached, n of each group = 45). Specimens in the nonbleaching control group were immersed in distilled water and stored at room temperature for 2 weeks. The second group underwent bleaching, and the surface of specimens was exposed to 15% carbamide peroxide (Opalescence PF15%) for 7 h a day for a total of 14 days. After 7 h of daily exposure to the bleaching agent, the specimens were rinsed and stored in distilled water at room temperature until the next day.

Colorimetry 2: After bleaching and before staining At the end of the 2 weeks, the specimens in both groups underwent colorimetry according to CIE L* a* b* color space as explained earlier.

Staining

Each "bleached/nonbleached" group in each of the 3 composites (n = 15 specimens per composite) was randomly divided into three groups (n = 5 per composite), and each group was exposed to one of the following staining solutions for 2 weeks: Cola (Coca Cola, USA); red grape juice (San Ich, Tehran, Iran); coffee: 5 g (equal to one teaspoon) of instant coffee (Nescafe Classic, Nestle, Istanbul, Turkey) was immersed in 200 cc of boiling water.

Colorimetry 3: After staining

The L*, a*, and b * color parameters were measured for each specimen by a spectrophotometer as explained earlier according to CIE L* a* b* color space.

Outcome

The changes in each color parameter in each session compared with the baseline were also calculated. These were used to calculate the ΔE using the following formula:

$$\Delta E = \sqrt{\left[\left(\Delta L^{*}\right)^{2} + \left(\Delta a^{*}\right)^{2} + \left(\Delta b^{*}\right)^{2}\right]}$$

 ΔE_{ab} values >3.7 indicated clinically perceivable color changes in this study.

Statistical analysis

Descriptive statistics and 95% confidence intervals were calculated for ΔE values. After testing the data normality using histograms as well as Kolmogorov– Smirnova and Shapiro–Wilk tests, ΔE values were compared among the groups using a two-way analysis of variance (ANOVA) followed by a Tukey *post hoc* test as well as a one-way ANOVA followed by a Dunnett's T3 test. Furthermore, an independent-sample *t*-test and a Mann–Whitney *U*-test were used for comparisons. The used software was SPSS 25 (IBM, Armonk, NY, USA). The level of significance was set at 0.05.

RESULTS

The two-way ANOVA showed that there was a significant difference among the ΔE values of 3 different staining agents' (P < 0.001). It also showed that there was a significant difference among the ΔE values of 6 combinations of composite types and bleached/nonbleached states (P < 0.001). However, the interaction of these factors was not significant (P = 0.294), meaning that the staining power of different agents was similar for different composites. The Tukey post hoc test showed that the results of each staining agent were significantly different from the results of two other staining agents [Table 2]. Moreover, several significant differences between some composite type + bleaching combinations [Table 2]. Nevertheless, bleaching did not change ΔE values in each of the 3 composite types [Table 2].

Subgroup analyses 1: Microhybrid composite

The *t*-test showed that bleaching had no significant effect on the stainability of microhybrid composite specimens (P = 0.236). In addition, according to *t*-test, bleached and nonbleached microhybrid composite specimens had no significant difference in color stability and stainability [P = 0.458, Table 3].

However, one-way ANOVA and Dunnett's T3 test showed a significant difference in the effects of staining solutions on the color of microhybrid composite specimens, such that coffee caused the greatest color change compared with cola and

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Table 2: The results of the Tukey post hoc test comparing ΔE values

Group A versus Group B	Difference	95% CI	Р
Cola versus red grape juice	-3.680	-5.4241.935	<0.001
Cola versus coffee	-6.773	-8.5175.028	<0.001
Red grape juice versus coffee	-3.093	-4.8381.348	<0.001
Microhybrid-nonbleached versus nanohybrid-nonbleached	-4.478	-7.4961.459	0.001
Microhybrid-nonbleached versus nanofilled-nonbleached	-4.450	-7.4691.432	0.001
Microhybrid-nonbleached versus microhybrid-bleached	0.763	-2.255-3.782	0.976
Microhybrid-nonbleached versus nanohybrid-bleached	-2.061	-5.079-0.9577	0.353
Microhybrid-nonbleached versus nanofilled-bleached	-2.052	-5.070-0.9667	0.358
Nanohybrid-nonbleached versus nanofilled-nonbleached	0.027	-2.991-3.046	1.000
Nanohybrid-nonbleached versus microhybrid-bleached	5.241	2.223-8.259	<0.001
Nanohybrid-nonbleached versus nanohybrid-bleached	2.417	-0.6014-5.435	0.190
Nanohybrid-nonbleached versus nanofilled-bleached	2.426	-0.5924-5.444	0.187
Nanofilled-nonbleached versus microhybrid-bleached	5.214	2.195-8.232	<0.001
Nanofilled-nonbleached versus nanohybrid-bleached	2.390	-0.6287-5.408	0.200
Nanofilled-nonbleached versus nanofilled-bleached	2.399	-0.6197-5.417	0.197
Microhybrid-bleached versus nanohybrid-bleached	-2.824	-5.842-0.1944	0.080
Microhybrid-bleached versus nanofilled-bleached	-2.815	-5.833-0.2034	0.082
Nanohybrid-bleached versus nanofilled-bleached	0.009	-3.009-3.027	1.000

CI: Confidence interval

Table 3: Descriptive statistics and 95% confidence intervals for $\triangle E$ values in all subgroups (*n* of each subgroup=5)

Bleaching	Composite Microhybrid (Z250)	Stain Cola	Mean 1.398*	SD 0.752	Minimum 0.53	Maximum 2.41	95% CI	
Nonbleached							0.464	2.332
		Red grape	6.396	3.326	2.16	9.57	2.267	10.525
		Coffee	5.552	1.489	3.63	7.40	3.703	7.401
	Nanohybrid (Z-350XT)	Cola	5.497	3.722	2.34	11.76	0.876	10.118
		Red grape	9.508	4.939	5.40	15.85	3.375	15.641
		Coffee	11.774	2.022	9.50	14.86	9.264	14.284
	Nanofilled (Z-250XT)	Cola	5.473	3.683	2.29	11.81	0.900	10.046
		Grape juice	9.532	4.893	5.37	15.79	3.457	15.607
		Coffee	11.692	2.047	9.48	14.89	9.151	14.234
Bleached	Microhybrid (Z250)	Cola	1.156*	0.233	0.91	1.45	0.866	1.446
		Red grape	3.300*	1.290	2.07	4.83	1.698	4.902
		Coffee	6.600	1.310	5.19	8.63	4.974	8.226
	Nanohybrid (Z-350XT)	Cola	2.277*	1.166	0.84	3.43	0.829	3.726
		Grape juice	5.709	3.804	2.66	11.27	0.986	10.432
		Coffee	11.542	2.318	8.79	14.60	8.664	14.421
	Nanofilled (Z-250XT)	Cola	2.264*	1.171	0.82	3.39	0.810	3.719
		Grape juice	5.697	3.784	2.58	11.31	0.999	10.396
		Coffee	11.540	2.296	8.84	14.57	8.688	14.391

*Mean ΔE values below 3.7, which are considered not perceptible. SD: Standard deviation; CI: Confidence interval

grape juice in both bleached and nonbleached composite specimens. Cola caused the smallest staining [Table 3 and Figure 1].

Subgroup analyses 2: Nanohybrid composite

The Mann–Whitney test indicated that bleaching does not influence color of nanohybrid composite (P = 0.683). Further, the Mann–Whitney showed that there is no difference between the color stabilities of bleached and nonbleached nanohybrid composite [P = 0.146, Table 3].

Yet, the one-way ANOVA and Tukey HSD indicated a significant difference in the influence of coloring solution on nanohybrid composite, such that coffee has had the highest effect on bleached and nonbleached nanohybrid composite compared with cola and red wine. Cola caused the smallest staining [Table 3 and Figure 1].

Subgroup analyses 3: Nanofilled composite

The Mann–Whitney test showed no effect of bleaching on the color of nanofilled composite (P = 0.652).



Figure 1: Means and 95% CIs for ΔE values in all 18 study subgroups (n = 5 per subgroup). CIs: Confidence intervals.

Further, according to the Mann–Whitney test, there was no difference in the color stability of bleached versus nonbleached nanofilled composite [P = 0.215, Table 3].

Nevertheless, the one-way ANOVA and Tukey showed a significant difference among the effects of different coloring solutions on nanofilled composite, such that coffee had the greatest effect on bleached and nonbleached nanofilled composite compared to cola and red wine. Cola caused the smallest staining [Table 3 and Figure 1].

DISCUSSION

Yu *et al.*^[18] discussed that composite resins are more susceptible to staining after bleaching compared with before bleaching. They attributed this heightened staining susceptibility to surface changes in composite following exposure to a bleaching agent. Some other studies stated that the effect of bleaching agents on the surface of composite restorations depended on the type of applied bleaching agent and duration of exposure.^[22,23]

Scanning electron microscopic and profilometric findings have demonstrated that 10%–16% concentrations of carbamide peroxide can significantly increase the surface porosities of microfilled and hybrid composite resins.^[19,20] Nonetheless, a previous study evaluated the effect of 6% hydrogen peroxide on a hybrid composite with interrupted periods of saliva storage and showed that saliva can form a protective barrier and decrease the effect of hydrogen peroxide on the surface properties of

restorative materials.^[34] Bailey and Swift^[21] indicated that 10% carbamide peroxide used during a 4-week protocol not only increased the surface roughness of restorative materials but also caused cracks on the surface of microfilled composite specimens. Wang et al.[24] showed that the effect of bleaching agents on the surface of different restorative materials may be variable depending on the duration of exposure and type of bleaching agent applied. In their study, different bleaching agents had different effects on each composite resin. In the present study, 15% carbamide peroxide (Opalescence) was used, which has a pH of 6.7. This pH is not acidic, and therefore, cannot roughen the composite surface. The short duration of exposure may be another reason for no significant effect of this bleaching agent on the composite surface. The exposure time was longer in studies that reported significant surface roughening of composite specimens by carbamide peroxide.^[21,34] In the present study, 15% carbamide peroxide had no significant effect on the color of composite specimens.

A study showed that the application of 15% hydrogen peroxide and warm 30% hydrogen peroxide caused a color change in composite resins with ΔE values ranging from 2 to 11.^[28] Li et al.,^[29] reported significant changes in color of nanohybrid packable composite after exposure to 15% carbamide peroxide. Another study demonstrated that high concentrations of hydrogen peroxide (30%-35%) can cause color change in materials with a highly resinous matrix.^[30] However, a more recent study concluded that surface roughness and gloss of the microhybrid and nanohybrid composites may not be influenced by bleaching.^[2] Rodrigues et al.^[32] as well found no significant discoloration as a result of bleaching using 35% hydrogen peroxide and 16% carbamide peroxide.^[32] Furthermore, Mohammadi et al.^[33] reported no clinically detectable discolorations in a microfilled composite and a giomer after bleaching with 15% carbamide peroxide, despite increases in their surface roughnesses.^[33] It should be noted, however, that they did not use any staining solutions.^[33] The differences in the results can be related to multiple methodological factors such as the type and duration of bleaching, the composites in use, or the method of testing color stability, the existence or lack of any staining solutions or their types if used, among others. Overall, according to a systematic review published in 2022,^[1] bleaching might cause statistically significant changes to the color stability of resin-based restorations, but these changes may not be clinically perceptible.

In general, the color change of restorative materials is attributed to the oxidation of superficial pigments and the amine content, which are also responsible for color change of composite resins over time. The degree of conversion is another influential factor on color stability of composite resins in contact with bleaching agents. Considering all the above, greater color change is expected in self-cure compared with light-cure composite resins.^[35] Results of the three composites were rather similar in terms of the staining potential of the staining agents. In this study, Filtek Z250 microhybrid composite experienced different levels of color change following immersion in different staining solutions. In the present study, coffee had a greater effect than cola and grape juice on color of composite specimens. Regarding Filtek Z-350XT nanohybrid at different coloring solutions, this study showed different color stabilities, with coffee having the highest effect than other coloring materials. In the present research, coffee, red wine, and cola have had the highest effect, respectively. Similarly, Bagheri et al.[25] reported that coffee caused the maximum color change. Another study showed equal color change by coffee and tea,^[26] and Bullem et al.^[27] demonstrated greater color change by tea than coffee. In the present study, coffee caused maximum color change of composite specimens followed by grape juice and then cola. Moreover, we observed that coffee always caused perceptible staining ($\Delta E > 3.7$), regardless of the composite in use or its bleaching history. Red grape juice caused such perceptible colorations in most cases. Cola did not cause any perceptible case in most cases.

This study was limited by some factors. It was *in vitro* and hence, not directly generalizable to clinical conditions; for instance, 2 weeks of continuous immersion in staining solutions does not resemble the use of staining solutions in real life. This is of course a limitation of any *in vitro* studies. Therefore, future clinical studies are warranted to verify our results. Furthermore, although the sample of this study was large compared to relevant studies, it would be better to precalculate the sample size before beginning the experiments.

CONCLUSION

This 3-phase study showed that bleaching might not alter the colorability of microhybrid, nanohybrid,

and nanofilled composites. Moreover, it was shown that coffee is unanimously the most potent stainer while cola is the weakest staining agent, regardless of the composite type in use and also regardless of prior bleaching or lack of it. Coffee always caused perceptible staining, regardless of the composite in use or its bleaching history. Red grape juice caused such perceptible colorations in most cases. Cola did not cause any perceptible case in most cases.

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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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