Original Article
The effect of dental water jet application on softened human enamel surfaces: A preliminary in vitro study using swept-source optical coherence tomography

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Received September 14, 2021; Accepted November 29, 2021; Epub January 15, 2022; Published January 30, 2022

Abstract: Objectives: To evaluate the surface loss of enamel, following water jet application, on acid-induced softened human enamel surfaces. Methods: Ninety enamel windows of 2×4 mm² were prepared on 45 extracted human premolar teeth. The specimens were divided into 2 groups: Group 1 (n=45) exposed to distilled water; Group 2 (n=45) exposed to citric acid (pH 3.2) for 30 minutes. All specimens were then subjected to a water jet for 20 seconds. Surface microhardness (SMH) and enamel thickness measurements were taken at baseline (t₁), post-distilled water/post-erosion (t₂) and post-water jet (t₃). Enamel thickness measurements were derived from the depth-resolved intensity profile (A-scans) of swept-source optical coherence tomography (SS-OCT) and SMH was used as a measurement of the state of mineralization. Repeated measures ANOVA was used to analyze the changes in SMH and enamel thickness (D) (α=0.05). Results: For Group 2, at t₂, there was a 54.28% and 14.14% reduction in SMH and D, respectively. Repeated measures ANOVA showed that the aforementioned mean reduction in SMH and D were statistically significant (P<0.05) at t₂ compared to t₁ for Group 2. However, at t₃, no significant difference (P>0.05) was observed in both measurements, compared to t₂ for both groups. Conclusion: Within the limitations of the study, the application of a water jet may not affect the thickness of enamel softened by acidic conditions.

Keywords: Water jet, initial enamel erosion, softened enamel, optical coherence tomography, microhardness

Introduction

Dental erosion is the loss of dental hard tissue, associated with extrinsic acid (for example in sports drinks and fruit juices) and/or intrinsic acid (gastric acids) that is not produced by bacteria [1]. The early stage of enamel dissolution is characterized by softening of the enamel surface [2]. This softening process happens when the enamel layer loses minerals from a layer extending between 0.2 and 5 µm below the tooth surface [3-5]. As softening progresses further into the enamel, it may result in substantial loss of minerals in the most superficial layer where this layer is lost completely [6]. Thus, further progressive loss will eventually lead to subsequent exposure of the underlying dentine, dentine sensitivity, and aesthetic problems [7].

The oral environment plays an extremely significant role in the wear behaviour of teeth during the erosion process. Saliva is the main component of the chemistry of the human mouth. Normally, saliva is neutral (pH 7), however, acidic agents can be introduced into the mouth [9]. The mouth environment of a person with a particularly acidic diet could be acidic with a pH of 3 [9], and the pH values of acidic drinks may range from 1 to 6.

In vitro and in situ studies of initial erosion have used acidic challenges consisting of either plain citric acid, various acidic beverages such as soft drinks (Coca Cola or Sprite: pH 2.3-3.2), juices (orange, grapefruit, lemon or blackcurrant: pH 3-4), wines (pH 2.9-4.2), acidic candies (pH 2.3-3.1) or sprays (pH 1.9-2.3) [8-10]. Among those cited acids, special attention has
been given to citric acid, since it is commonly found in citric fruits and juices [11].

Enamel softening is however reversible, whereby salivary calcium and phosphate have been reported to remineralize the softened enamel [12]. It was shown that the abrasion resistance of eroded enamel can be improved when exposed to artificial saliva (in vitro) [12] and to a smaller degree, to the oral environment (in situ) [4, 12], which means that the enamel softening stage can be reversed and have the potential to re-harden [13]. The clinical manifestation of enamel erosion, therefore, may be the result of the removal of the softened surface before the remineralizing action of saliva [14].

Intra-orally, erosion does not occur exclusively where the chemical dissolution occurs simultaneously with mechanical forces [15, 16]. As minerals are released during erosion, the mechanical and physical properties of this softened enamel are modified and are more susceptible than sound enamel to mechanical wear such as attrition and abrasion [12, 17]. Attrition is the gradual loss of hard tooth substances from occlusal contacts with an opposing dentition or restorations [18], and this can be caused by extrinsic factors such as parafunctional habits of bruxism, traumatic occlusion in the partially edentulous dentition, and malocclusions [19]. Zhang et al. [20] reported that in acidic environments, the softened enamel layer becomes relatively smooth following enamel-on-enamel (attrition) wear.

Softened enamel has also been reported to be prone to abrasion [21]. Abrasion is the loss of tooth substance through mechanical means, other than tooth contact [22]. The most common cause of dental abrasion is tooth brushing and the severity and distribution of tooth brushing abrasion wear may be related to brushing technique, time, frequency, bristle design, and the abrasiveness of the dentifrice [18, 23, 24]. However, without the presence of an acid, neither tooth brushing alone nor tooth brushing with toothpaste has been shown to cause wear of enamel [25]. Thus, tooth brushing following the consumption of acidic beverages is recommended to be postponed to minimize or avoid enamel loss [25].

Apart from tooth brushing, softened enamel may also be worn by abrasion from soft tissues including the tongue [26-28] and buccal mucosa during mastication and swallowing [29]. The palatal surfaces of the upper teeth are areas known to be constantly subjected to shear forces either from the keratinized dorsum of the tongue during speech and swallowing or through food mastication (occlusal surfaces) [29]. Abrasive action from ultrasonication with water [30] has also been reported to be able to remove the softened surface. Wiegand et al. [3] reported that enamel loss after 30-second ultrasonication with water seems to match abrasion after 20 brushing strokes with toothpaste slurry or 50 brushing strokes with distilled water.

The dental water jet (also known as an oral irrigator or water flosser) is designed to remove plaque and soft debris through the mechanical action of a jet stream of water and has been used as an adjunct device to tooth brushing. It has been reported to be effective in eliminating biofilm from tooth surfaces and the reduction of subgingival pathogenic bacteria from pockets as deep as 6 mm with the use of water flossing [31-33]. With regards to its mechanism of action, the dental water jet works by directly applying a pulsed stream of water to the dental plaque biofilm [34]. Research has shown that the production of 1,200 to 1,400 pulsations per minute with a pressure range of medium to high or 50 psi to 90 psi produced the best results in plaque removal [35]. A study at the University of Southern California found that a 3-second treatment of pulsating water (1,200 pulses per minute) at medium pressure (70 psi) removed 99.9% of plaque biofilm from treated areas [33]. Therefore, it might be speculated that due to the amount of pressure exerted from the water jet onto the softened surface, a certain amount of the demineralized layer might be removed.

Taking into account that to date, there is no report on whether a water jet potentially removes acid-induced softened enamel, thus the main objective of this study is to evaluate the surface loss of enamel following the application of a dental water jet on acid-induced softened human enamel surfaces. The null hypothesis of this study was that the application of a dental water jet on acid-induced softened human enamel surfaces does not cause enamel surface loss.
Softened enamel, swept-source optical coherence tomography

Materials and methods

Preparation of enamel specimens

Forty-five extracted human premolar teeth were collected from various clinics, which belonged to various age and race groups. Teeth were stored in 2% Chloramine-T solution to inhibit bacterial growth for two weeks and subsequently in distilled water at 4°C to ensure hydration until use. The crowns of the teeth were sectioned into buccal and lingual halves with a water-cooled diamond sectioning machine (Micracut@125, Metcon). Ninety sound buccal or lingual enamel surfaces were obtained and embedded in cold-cure epoxy resin (Mirapox 950-230 A/B, Miracon Sdn Bhd) in custom-made rubber molds to form a 15 mm x 12 mm x 6 mm rectangular-shaped disc, exposing an enamel area of approximately (X, Y) 2 mm x 4 mm. The exposed enamel areas were polished on a Buehler Isomat II polishing machine using 600-grit silicon carbide paper (Buehler, Lake Bluff, IL, USA), followed by 6, 3, and 1 µm diamond abrasive powder (Buehler, Metadi diamond spray) on Buehler polishing cloth under constant cooling. After polishing, the smear layer was removed by ultrasonication. The specimens were ultrasonicated for 50 seconds in a stainless-steel container filled with distilled water [36]. Then, baseline (t₁) surface microhardness (SMH) measurements and swept-source optical coherence tomography (SS-OCT) scanning were performed. The specimens were divided into 2 groups: Group 1 (n=45) (Control Group), where specimens were exposed to distilled water; and Group 2 (n=45) where specimens were exposed to citric acid.

Surface softening

The rectangular-shaped resin blocks containing the enamel specimens were suspended in a beaker containing 150 ml of 0.3% citric acid (A&C American Chemicals) at pH 3.2. The beaker was placed on a magnetic stirrer (IKA RCT basic) set to 275 rpm to ensure that all specimens were treated equally during the acid-immersion. The pH of the acid was monitored with a pH meter and the temperature of the solution was kept constant at 36°C throughout the experiment. After 30 minutes, the specimens were removed from the acid and rinsed under a reservoir of running water for 1 minute to remove excess acid. SMH measurements, and SS-OCT scanning were subsequently performed (post-erosion (t₂)).

Dental water jet

A triple syringe, attached to a portable cutting unit (Forest Medical), was used to deliver streams of water from the water jet onto the enamel specimens, with oil-free compressed air. The tip of the syringe was placed 1 cm from the enamel surface at an approximately 90 degrees angle and the water jet was delivered for 20 seconds [37] at a pressure of 70 psi [33]. The SMH measurements and SS-OCT scanning were then repeated, post-water jet (t₃). A flowchart of the study design is shown in Figure 1.

Measurements

All specimens underwent SMH measurements and SS-OCT imaging at baseline (t₁), post-erosion (t₂), and post-water jet (t₃).
Surface microhardness

SMH measurements were performed using the microhardness indenter (HMV-2000, Shimadzu) as a measure of enamel softening/demineralization. The specimens were inserted into a customized jig that was positioned parallel to the stage to minimize sliding of the indenter during loading. The jig was also used to obtain a reproducible position during all measuring time-points. The enamel specimen was placed parallel to the stage prior to indentation and approximately 1 mm² of surface area was identified for indentation. Micro-indents were made using a Knoop diamond indenter, under a static load of 50 g applied for 5 seconds [38, 39] to obtain the Knoop Hardness Number (KHN). The apparatus was recalibrated before each use. Five indentations were made on each specimen approximately 100 μm apart at the identified area and the mean KHN was then calculated. The width of each indentation was measured and the KHN was generated from the HMV-2000 software, based on the width of each indentation. The outcome measure was expressed as the percentage of SMH change (ΔSMC), calculated based on the differences between KHN at t₁, and the subsequent time points. ΔSMC was calculated as:

$$\Delta SMC(t_2) = 100 \left[ \frac{KHN(t_2) \cdot KHN(t_1)}{KHN(t_1)} \right]$$

KHN (t₁) = KHN at Baseline
KHN (t₂) = KHN Post-Erosion
t₁ = Baseline
t₂ = Post-Erosion

$$\Delta SMC(t_3) = 100 \left[ \frac{KHN(t_3) \cdot KHN(t_2)}{KHN(t_2)} \right]$$

KHN (t₂) = KHN Post-Erosion
KHN (t₃) = KHN Post-Water jet
t₂ = Post-Erosion
t₃ = Post-Water jet

Enamel thickness

SS-OCT data acquisition: A Swept-source OCT Imaging System (OCS1300SS, Thorlabs, UK) was used to capture cross-sectional images of the enamel specimens. The instrument incorporates a broad-band, frequency-swept near infra-red source centered at 1325 μm. The imaging probe was attached to a stand and the specimens were placed in a customized positioning jig which was attached to a translational stage that allows X, Y translation, and Y, Z rotation. During scanning, the specimens were placed on the translational stage of the SS-OCT system. The stage was fixed with the repositioning jig so that each scan was performed at the same position and alignment during different measuring time points. The jig was used to ensure that each scan was performed at the same position and alignment during different measuring time points. The repeatability of the SS-OCT scan was preserved at consecutive measuring time points. The specimens were placed at the same orientation and alignment as accurately as possible, and the B-scan was performed along a line between the two points marked by a marker pen on the specimen surface. Just prior to SS-OCT scanning, specimens were dried with oil-free compressed air at a pressure of 29 psi administered from a point 5 cm from the tooth surface using a three-way syringe for 10 seconds [40]. SS-OCT images were acquired, and backscattered light intensity as a function of depth was analyzed for each time point [41].

The Thorlabs SS-OCT capturing software (Swept Source OCT Imaging System Version 2.3.1, Thorlabs) was used to capture the image, configure the SS-OCT settings and guide the light beam. The scanning beam was configured to scan an area of 2 mm x 4 mm window in the X-Y direction and at a depth of 3 mm in air, corresponding to an axial physical depth of 1.85 mm (Z-axis) in enamel (refractive index =1.62), with a resolution of 1024, 512 and 512 pixels at the X, Y and Z axis respectively. Therefore, a total of 512 SS-OCT cross-sectional scans (B-scans), approximately 30 μm apart, were attained for each investigation site. The B-scans were captured on a logarithmic scale and saved in the Large DR3 color map of the image capturing software. The brightness and contrast were configured to cover an intensity range between -36 dB and -10 dB.

All SS-OCT data were post-processed with a 2-dimensional OCT MATLAB analytics program (MathWorks Inc.) [36]. SS-OCT B-scan images
were loaded, and a similar region of interest (ROI) was selected for each specimen for all time points. The ROIs have a dimension of 1 mm x 2 mm (X, Y) and were located in the middle of the exposed area. The ROIs were then subjected to a surface determination and alignment algorithm and a mean depth-resolved intensity profile (A-scan) for each specimen was generated, and the intensity values were exported automatically to Excel sheets. The enamel specimens were scanned at pre-erosion \( t_1 \), post-erosion \( t_2 \) and post-water jet \( t_3 \). In an attempt to reduce variations in the selection of the b-scans to be measured, a notch was prepared using a high-speed bur on the side to act as a reference point [42].

**Enamel thickness measurement:** The enamel thickness was measured using the mean A-scan generated from the ROIs. The first maximum intensity \( I_1 \) observed in the mean A-scan, indicates the change in reflectivity between background and enamel and was used to represent the enamel-air interface. The intensity of the reflected light attenuates exponentially thereafter as the light transmits through enamel until dentine is reached, where another obvious increase in intensity \( I_2 \) is observed. The location of \( I_2 \) for this study was defined as the highest intensity that immediately follows the lowest point of attenuation from \( I_1 \) (Figure 2). The thickness of enamel \( D \) was then determined by the distance between \( I_1 \) and \( I_2 \) [42]. The outcome measure used was the thickness of enamel \( D \) and also the percentage change of the enamel thickness \( \Delta D \). \( \Delta D \) was calculated as below:

\[
\Delta D (t_2) = 100 \left( \frac{D (t_2) - D (t_1)}{D (t_1)} \right)
\]

- \( D (t_1) = D \) at Baseline
- \( D (t_2) = D \) at Post-Erosion
- \( t_1 = \) Baseline
- \( t_2 = \) Post-Erosion

* denotes the lowest point of attenuation after the first peak \( (I_1) \).
Softened enamel, swept-source optical coherence tomography

Table 1. Mean SMH and D at baseline (t₁), post-distilled water (t₂) and post-water jet (t₃) for Group 1 (Control group)

<table>
<thead>
<tr>
<th></th>
<th>t₁ (n=45)</th>
<th>t₂ (n=45)</th>
<th>t₃ (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMH (KHN) (±SD)</td>
<td>251.75 (±66.08)</td>
<td>252.44 (±42.67)</td>
<td>250.25 (±49.12)</td>
</tr>
<tr>
<td>D (µm) (±SD)</td>
<td>379.60 (±79.95)</td>
<td>370.76 (±41.76)</td>
<td>368.04 (±43.27)</td>
</tr>
</tbody>
</table>

Within each row, distinct lower-case letters indicate significant differences among the time points (Repeated measures ANOVA, α=0.05).

Table 2. Mean SMH and D at baseline (t₁), post-erosion (t₂) and post-water jet (t₃) for Group 2

<table>
<thead>
<tr>
<th></th>
<th>t₁ (n=45)</th>
<th>t₂ (n=45)</th>
<th>t₃ (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMH (KHN) (±SD)</td>
<td>268.47 (±96.08)</td>
<td>122.74 (±32.97)</td>
<td>124.29 (±50.09)</td>
</tr>
<tr>
<td>D (µm) (±SD)</td>
<td>389.90 (±79.95)</td>
<td>334.76 (±39.79)</td>
<td>345.05 (±45.07)</td>
</tr>
</tbody>
</table>

Within each row, distinct lower-case letters indicate significant differences among the time points (Repeated measures ANOVA, α=0.05).

\[
\Delta D (t₃) = \frac{100}{D (t₂)} \cdot \frac{D (t₂) - D (t₃)}{D (t₂)}
\]

\[D (t₂) = D \text{ Post-Erosion}\]

\[D (t₃) = D \text{ Post-Water jet}\]

\[t₂ = \text{Post-Erosion}\]

\[t₃ = \text{Post-Water jet}\]

Statistical analysis

Data were analyzed using the IBM SPSS Statistics for Windows software Version 25.0 (IBM Corporation). All statistical analyses were carried out at a significance level of \(\alpha=0.05\). Data from surface microhardness (KHN) and enamel thickness (D) presented a normal and homogeneous distribution and were subjected to repeated measures analysis of variance (ANOVA). Bonferroni post-hoc tests were subsequently performed to detect any significant difference before and after each time point for both measurements.

Results

Tables 1 and 2 show the mean SMH and D at (t₁), (t₂), and (t₃) for both Group 1 and 2, respectively. For Group 1, there was no significant difference in SMH and D (P>0.05) between time-points. For Group 2, at t₁, the mean SMH was 268.47 (±96.08) KHN and the mean D obtained from SS-OCT A-scans was 389.90 (±79.95) µm. At t₂, the mean SMH and D were 122.74 (±32.97) KHN and 334.76 (±39.79) µm, respectively. At t₃, the mean SMH and D were 124.29 (±50.09) KHN and 345.05 (±45.07) µm, respectively. Repeated measures ANOVA showed a significant difference in SMH and D (P<0.05), at t₂, while there was no significant difference in both SMH and D at t₃ (P>0.05).

Table 3 shows the results of repeated measures ANOVA for SMH and D, showing the P-values between time points. The average baseline thickness and reduction after erosion were 389.90 (±79.95) µm and 55.14 (±24.8) µm respectively, which means the \(\Delta D\) at t₂ was 14.14% (Table 4). The \(\Delta SMH\) (t₂), and \(\Delta D\) (t₃) were 1.26 (±39.26)% and -3.07 (±57.07)%, respectively.

Discussion

This study evaluated the enamel surface loss of acid-induced softened enamel surfaces on human teeth, following the application of an awater jet. Knoop microhardness measurements were used as the parameter to evaluate the softening of enamel that occurred after the acid challenge. It has been shown to be sensitive in evaluating early de- and remineralization in the outermost layer of enamel [43]. Citric acid is a common fruit acid found in fruit juices and soft drinks and it has been used in many published dental erosion studies, as reviewed by de Carvalho et al. [44]. The erosive effect of citric acid in this study was confirmed by a significant decrease of approximately 54% in mean KHN value, resulting in a mean SMH of 122.74 (±32.97) KHN.

The human enamel is often thickest at the cusp, up to 2.5 mm, and thinning down to almost a knife-edge at the cemento-enamel junction (CEJ) [45]. The thickness of the enamel specimens used in this study was relatively thin (0.2-0.5 mm) because the specimens had to be polished flat for the measurement of SMH. A mean reduction of 55.14 (±24.8) µm in enamel thickness which was approximately

SS-OCT is an imaging system with functions similar to ultrasound, but only uses light rather than sound. It is a technique used to produce non-invasive, high-resolution images of biological microstructure [51]. It can obtain cross-sectional imaging by measuring the magnitude and echo time delay of the backscattered light by using a broad-band light source.

Several studies have used SS-OCT to measure enamel thickness [42, 52, 53] either by visually assessing the cross-sectional images (B-scans) or by using the depth-resolved intensity profile (A-scans). Wilder-Smith et al. [52] monitored over 3 weeks the erosive wear of patients having Gastroesophageal Reflux Disease (GERD) in vivo, by measuring the distance of the enamel surface to the DEJ on the SS-OCT B-scans. They found that the imaging depth was more than adequate to measure the full enamel thickness and the DEJ served well as an accurate baseline reference. However, Chan et al. [53] when attempting to measure enamel thickness of eroded enamel in vitro with PS-OCT, found that the increase in scattering due to subsurface demineralization or surface roughness limited the ability to resolve the DEJ. This particular problem was not encountered in this study. The contradictory findings between Wilder-Smith et al. [52] and Chan et al. [53] were most likely due to the different severity of the subsurface demineralization in these two studies. The level of demineralization in vivo is known to be lower than that induced in vitro due to the remineralization effect of saliva. In addition to that, Chan et al. [53] exposed soft eroded surface. In order to measure tooth surface loss due to erosion, both contact and non-contact profilometry require a reference area that is not affected by erosion. However, with profilometry, the depth of softening on eroded enamel surfaces may not be measured [50].

Surface profilometry has been extensively used to characterize enamel loss caused by erosion. The surface of a specimen is scanned to produce a two-dimensional or three-dimensional profile, using either a contact or a non-contact measuring device [47]. In contact profilometry, the surface is scanned using a stylus with a diamond or steel tip [48, 49]. It is the most applied method to measure tooth loss, despite its main limitation of potential tissue damage [50]. Non-contact profilometry on the other hand uses a laser light probe, thus there is no direct physical contact between the probe and the surface, and no damage occurs to the soft eroded surface. In order to measure tooth surface loss due to erosion, both contact and non-contact profilometry require a reference area that is not affected by erosion. However, with profilometry, the depth of softening on eroded enamel surfaces may not be measured [50].
their samples to acid for 12 hours which would have resulted in severe demineralization which in turn have caused high scattering at immediate subsurface.

Recently, Algarni et al. [42] measured enamel thickness with OCT A-scans by measuring the distance between the coordinates of the first peak and that of the second peak, where the first peak represents the enamel surface and the second peak being the peak that indicated the most evident change in reflectivity between enamel and dentin, near the DEJ area. They validated it against micro-computed tomography and histology and found good agreement between the measurements made with this three equipment. However, they noted that there was some degree of subjectivity in the protocol of determining the second peak. In order to reduce this subjectivity, an additional criterion was added in this study where the second peak ($I_2$) is established as the peak that immediately follows the lowest point of attenuation after the first peak ($I_1$) (Figure 2).

The concept of the water jet used in this study was based on the usage of an oral irrigator as an adjunct to tooth brushing for plaque control and it is designed to remove plaque and soft debris through the mechanical action of a jet stream of water of set pressure. However, within the limitation of the study, the application of the water jet was used instead of using a commercially available product such as a Waterpik or other water flossing devices. Furthermore, the water jet was applied in a shorter time, thus the result may not necessarily be extrapolated to an in vivo environment. The pressure exerted from the unit varies between the water jet devices, ranging from 10-120 psi [46, 54]. Kato et al. [46] reported that approximately 85% of plaque biofilms could be removed by vertical irrigation with water pressures exceeding 50.8 psi. In this study, there was no significant difference in $D$ at $t_3$ ($P > .05$). Therefore, the null hypothesis that there is no significant enamel loss following the application of a dental water jet on acid-induced softened human enamel surfaces was accepted.

It is known that acid softened enamel has a heightened risk of being abraded and attrited. An in-situ study reported that softened bovine enamel surface was easily removed when subjected to mechanical abrasive forces from tooth brushing [55], whilst another in situ study showed that the mean surface loss of softened human enamel specimens could reach 0.258 µm after abrasion [4]. However, no one to date has reported on the effect of a water jet on the softened enamel surface. A 30-min acid exposure was chosen for this study, in order to make sure that signals will be picked up in OCT, although it has been reported that changes could already be seen after 10 min of acid challenge [56].

**Conclusion**

Overall, the results of this study revealed that the acid challenge significantly reduced the surface microhardness, and the enamel thickness. However, the application of a water jet on eroded enamel did not significantly affect the surface microhardness nor the thickness of enamel. The data presented here indicates that the application of a dental water jet may not further remove the already eroded and softened enamel, but further study is needed to confirm this. Within the limitations of the current study, it can be concluded that, in vitro, the application of water jet may not be contraindicated for patients diagnosed with erosive toothwear.

**Acknowledgements**

The authors would like to thank HP Chew and CM Zakian for their contributions. The work was supported by the University of Malaya and High Impact Research Grant, Ministry of Higher Education, Malaysia (UM-MOHE HIR Grant UM.C/HIR/MOHE/ DENT/11).

**Disclosure of conflict of interest**

None.

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**References**


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