

Effect of oral exposure on chemical, physical, mechanical, and morphologic properties of clear orthodontic aligners

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Introduction: The dental industry is heavily committed to developing more esthetic solutions for orthodontic treatments. Invisalign is a system of transparent orthodontic aligners introduced as an alternative to conventional orthodontic fittings with brackets and metal wires. This study aimed to assess the chemical, physical, mechanical and morphologic changes in these polymeric aligners after exposure to the oral environment. **Methods:** Twenty-four Invisalign orthodontic aligners were equally divided into 2 groups: an in vivo aged group in which patients used aligners for 14 days and the reference group, unexposed to the oral environment. Different experimental techniques were used to study the chemical structure, the color changes and translucency, the density and subsequent volume of the aligners, mechanical properties, surface roughness, morphology and elemental composition. The data were subjected to several statistical analyses. **Results:** Clear orthodontic aligners exhibit chemical stability but undergo a statistically significant optical change in color and translucency. There was a gradual increase in the water absorption rate and the dimensional variation of the polymer, indicating a strong correlation among these factors. The mechanical properties of the polymer showed a statistically significant decrease in its elastic modulus and hardness. There was a slight tendency toward increased surface roughness of the material, but no statistical differences were found between reference and aged groups. The surface morphology of the used aligners demonstrates microcracks, distortions and biofilm formation. **Conclusion:** Intraoral aging adversely affected the physical, mechanical, and morphologic properties of the Invisalign appliance. (Am J Orthod Dentofacial Orthop 2023;164:e51-e63)

Contemporary orthodontics has searched for more orthodontic appliances and accessories.¹⁻³ As a consequence, the dental industry has reasserted its commitment to develop more esthetic solutions for

orthodontic treatments and meet the expectations of social demand.^{4,5} As an outcome, a system of clear orthodontic aligners called Invisalign (Align Technology, Santa Clara, Calif) was introduced in 1998 for orthodontics as an alternative to conventional orthodontic fittings with brackets and metal wires. The purpose was to promote tooth movement using a removable device made with a thermoplastic polymer.⁶⁻⁸ The technique has since been accepted, especially by patients, and gradually by professionals.^{9,10}

Thermoplastic polymers were chosen to make transparent orthodontic aligners because of their viscoelastic and optical properties.¹¹⁻¹³ However, thermoplastic polymers are very sensitive to temperature, water and moisture, the chemical nature of the environment, oxygen and organic solvents, the time elapsed after elastic deformation, and the manufacturing process.^{14,15} Therefore, exposure to an adverse environment, such as the oral environment, could result in changes in the properties of these materials, which might compromise treatment efficacy.⁵

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Thus, this study aimed to evaluate the possible chemical, physical, mechanical and morphologic changes resulting from the aging of the constituent polymer of transparent orthodontic aligners after exposure to the oral environment for 14 days and after immersion in artificial saliva.

METHODS

The Institutional Board of the Research Ethics Committee of University Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, approved protocol no. 1.991.131 on April 5, 2017, and informed consent was obtained from study participants. The study samples consisted of 24 ($n = 24$) Invisalign transparent orthodontic aligners (Align Technology), divided equally into 2 groups: an in vivo aged (AG) and reference (RG) groups. The aligners that constituted AG were used for 14 days by 12 patients undergoing orthodontic treatment with clear aligners. Patients were instructed to use the appliance for 20–22 h/d, as recommended by the manufacturer. RG was composed of aligners not exposed to the oral environment; moreover, their parameters and characteristics were considered the manufacturing standard for this polymer, thereby enabling comparisons regarding the possible changes resulting from intraoral aging. All the analyses were distributed according to the region of the maxillary orthodontic aligner, as indicated by the illustrative diagram (Fig 1).

The changes in the chemical composition of the material were studied qualitatively by the attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) technique. The specimens were made measuring 4×4 mm, using the buccal surface of the aligner, corresponding to tooth 11. Then, they were washed in an ultrasonic tub and dried with paper towels. The spectra were obtained by positioning the buccal surface of the specimen so that it made contact with the diamond tip of a single reflection FTIR spectrometer (Platinum Alpha-ATR; Bruker, Billerica, Mass), under the following operating parameters: range of $4000\text{--}5000\text{ cm}^{-1}$, resolution of 4.0 cm^{-1} , and depths of analysis estimated at $2\text{ }\mu\text{m}$ in 1000 cm^{-1} . All spectra were analyzed and submitted to baseline corrections by Opus software version 7.5 (Bruker). The following wave numbers were chosen to enable observation of possible hydrogen bond-type intermolecular interactions, which can occur after intraoral exposure by water absorption: NH (3316 cm^{-1}), C = O (1698 cm^{-1}), C = O (1728 cm^{-1}), and C = O (1525 cm^{-1}).

In this analysis, only aligners not worn by patients were used. The specimens were prepared with a 7-mm

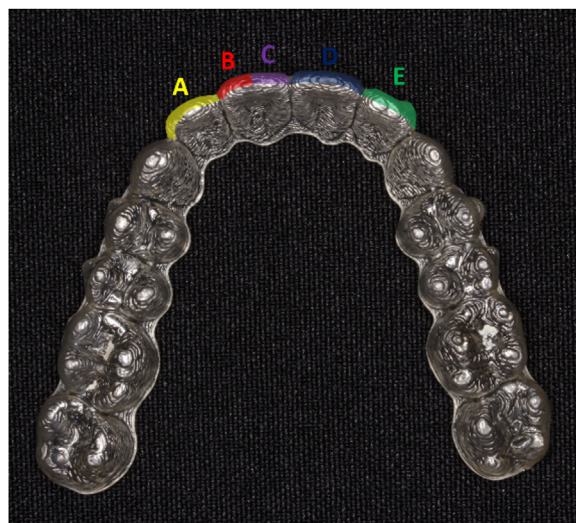


Fig 1. Illustrative diagram of the analysis distribution according to the region of the maxillary aligner: **A**, Chemical analysis by ATR-FTIR; **B**, Color analysis; **C**, Mechanical properties analysis; **D**, Morphologic analysis by OM, SEM, and EDS; **E**, Water absorption and volume change analysis. *ATR-FTIR*, attenuated total reflectance-Fourier transform infrared technique; *OM*, optical microscope; *SEM*, scanning electron microscope; *EDS*, energy dispersive spectroscopy.

diameter and a buccal surface corresponding to tooth 11. The specimens were weighed before the test using a precision analytical digital balance (XSE, Mettler-Toledo, Columbus, Ohio). The initial mass (M_0) was obtained for preset time points of 1, 3, 24, 48, 72, 168, and 336 hours. The Archimedes principle¹⁶ was used to obtain the specimen volume by placing each specimen in a holder coupled to a thermometer immersed in distilled water to obtain the initial mass weighed in distilled water (M_θ). The volumes of the specimens were obtained at each predetermined time interval by using the following formula, described in the equipment manual (XSE, Mettler-Toledo):

$$V = \alpha (M_a - M_w) / (\rho_0 - \rho_L)$$

where V is volume, α is the balance correction factor (0.99985), M_a is the mass weighed in air, M_w is the mass weighed in distilled water, ρ_0 is the density of the auxiliary liquid (distilled water), according to the temperature at which it was weighed, and ρ_L is the density of the air (0.0012 g/cm^3).

The following formula was applied to calculate the rate of water absorption:

$$W_A\% = (M_0 - M_1) \times 100 / M_0$$

where $W_A\%$ is the water absorption percentage, M_0 is the initial mass, and M_1 is the mass at each time interval. The specimens were first weighed and then immersed in artificial saliva and stored in an oven (Quimis, São Paulo, Brazil) at 37°C to enable simulation of the analyses to determine water absorption rate and volume change at the established periods. The values for water absorption rate, volume and volume variation (ΔV) were obtained for each time interval according to the following formula:

$$\Delta V = V_{\text{determined time}} - V_{\text{initial}}$$

The color changes and translucency were evaluated by a spectrophotometer (CM2600D, Konica Minolta; Chiyoda, Tokyo, Japan), according to the Commission Internationale de L'Eclairage color system: $L^*a^*b^*$ (CIE $L^*a^*b^*$),^{17,18} which represents the colors on the basis of the following parameters: L^* represents the luminosity or brightness, and ranges from 0 (black) to 100 (white); a^* represents the color scale ranging from red (positive values) to green (negative values); and b^* represents the color scale ranging from yellow (positive values) to blue (negative values). The specimens were made 7 mm in diameter from the buccal surface of the aligner, corresponding to tooth 11. They were placed in the spectrophotometer window, and measurements were made of parameters L^* , a^* and b^* on white and black spectrophotometric backgrounds. The total color variation (ΔE^*) was determined by the following equation^{19,20}: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^* , Δa^* , Δb^* were the differences among the L^* , a^* and b^* parameters of RG and AG. The translucency parameter (TP) was measured using the following equation²¹:

$$TP = [(L^*_B - L^*_W)^2 + (a^*_B - a^*_W)^2 + (b^*_B - b^*_W)^2]^{1/2}$$

where letters B (black) and W (white) represent measurements made of the same specimen on black and white spectrophotometric patterns, respectively. The ΔE^* value was expressed in clinical standards by converting ΔE^* to the scale developed by the National Bureau of Standards (NBS) (Fig 2). The ΔE value was converted by using the equation: $NBS = \Delta E^* \times 0.92$.²²⁻²⁴

The hardness (H) and elastic modulus (E) values of the polymeric aligners were obtained with the nanoindentation test using the method reported by Oliver and Pharr.^{25,26} The mechanical properties were assessed by fixing 4 × 4 mm buccal surface specimens corresponding to tooth 11 to a polyethylene base using epoxy glue (Araldite, Embu das Artes, Brazil). This set was then attached to a glass slide and glued with adhesive (CristalBond 509; Aremco, Clarkstown, NY) to a metal base suitable for making the indentations and held in place snugly to

prevent movement during the test. The tests were performed using a nanoindenter (Agilent G200, Santa Clara, Calif) with a displacement resolution of <0.01 nm and a force resolution of 50 nN. Ten indentations were performed for each specimen, after which the mean and standard deviation were obtained. The indentation positions in the polymeric aligners were defined using an optical microscope at 40× magnification attached to a nanoindenter. Conventional nanoindentation measurements were performed using Berkovich diamond tip area calibration, applying a maximum load of 3 mN, single-cycle loading of 15 seconds and holding the tip for 2 seconds at peak load. The assumed Poisson coefficient was 0.43.⁵ The hardness and modulus of elasticity were obtained according to the Oliver-Pharr method^{25,26} and International Organization for Standardization 14577-1 international standard specification, using NanoSuit 5 software (Agilent), as follows:

The elastic modulus (E) was obtained according to the following equation:

$$E = (1 - \nu)^2 \left[\frac{1}{E_r} - \frac{1 - \nu_i^2}{E_i} \right]^{-1}$$

where ν (0.43) and ν_i (0.07) are respectively the Poisson ratio of the sample and the indenter value, E_i is Young's modulus of the indenter (1140 GPa), and E_r is the reduced modulus as defined by the expression^{25,26}:

$$E_r = \frac{\sqrt{\pi}}{2S\sqrt{A}}$$

where A is the projected contact area of the indenter, and S is the stiffness of the material obtained from the slope of the initial portion of the discharge curve.

The hardness (H) is determined by the following equation:

$$H = P/A$$

where P represents the maximum load applied to the specimen.

These analyses evaluated surface morphology using an optical microscope (OM), scanning electron microscope (SEM), and semiquantitative identification of substances deposited on aligner surfaces by energy dispersive spectroscopy (EDS). An LSM 700 microscope (Zeiss, Oberkochen, Baden-Württemberg, Germany) was used with a 10× objective lens at 100× magnification. The specimens were fixed with carbon tape on an aluminum plate and gold-coated for SEM analysis. The analyses were performed using the JSM-6460 LV SEM (JEOL; Akishima, Tokyo, Japan) and the Thermo Scientific Noran System 200 EDS software. SEM was operated

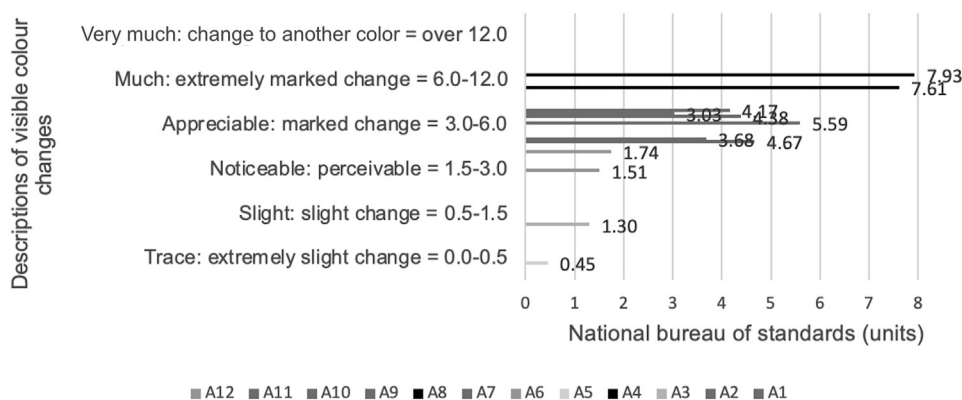


Fig 2. Orthodontic aligner distributions and classifications by NBS rates of visible color change after 14 days of exposure to the oral environment. *NBS*, National Bureau of Standards.

at 20kV, and readings were obtained at 8×, 500×, and 5000× magnifications.

The roughness parameters defined in this analysis complied with International Organization for Standardization 25178-2 standards. Roughness data were obtained and analyzed using the Form Talysurf 60i 3-dimensional (3D) profilometer (Taylor Hobson; Leicester, Leicestershire, United Kingdom) together with Ultra Software (Taylor Hobson) and Talymap 3D Analysis (Taylor Hobson). A central area of 1 mm² on each specimen was scanned with a resolution of 20 nm on the z-axis, 2 μm on the y-axis, and 4000 points on the x-axis. The reading speed was constant and equal to 0.5 mm/s. The specimens were attached with adhesive tape to the center of the reading platform, and a specified marking was made so that the reading would always be made in the center of each specimen. Three-dimensional images were reconstructed automatically with image reconstruction software (Talymap 3D Analysis; Taylor Hobson). The S_a (mean roughness) parameter is an extension of R_a (arithmetical mean height of a line), and was used to evaluate the surface roughness (S_a) of the material. This parameter corresponds to the arithmetic mean of roughness of the area analyzed, based on the average values between the peaks and valleys of the surface profile of the specimen.²⁷

Statistical analysis

The sample size of the study was calculated using an α significance level of 0.05 and a β significance level of 0.20, based on an initial pilot study with 10 specimens, 5 from AG and 5 from RG. The mean and standard deviations before (49.04 ± 0.66) and after (46.43 ± 2.74) oral environment exposure of the TPs were analyzed using G*Power software (version 3.9.1.2; Franz Faul

Universitat, Kiel, Germany).²⁸ This parameter was used to obtain the sample size to the detriment of the other analyses because it was observed that this analysis required the largest number of specimens to reach the test power and desired significance. The total sample was 22 specimens (11 for each group), which was later increased to 24, considering the possibility of sample loss (resulting in 12 specimens for each group). The results were analyzed statistically using SPSS software (version 22; IBM, Armonk, NY). Adherence to the normal curve was assessed by the Shapiro-Wilk test. The normal distribution was used to evaluate the values of the parameters of color change (L, a, and b), translucency (TP), and S_a before and after exposure to the intraoral environment. Analysis of variance of repeated measures with Bonferroni correction for several comparisons was applied to evaluate the water absorption rate and volume change parameters. The correlation between the $W_A\%$ and ΔV was made using the Pearson correlation coefficient, and a linear regression analysis was applied to mathematically express the relationship between the 2 variables. A significance level of 0.05 was established for all analyses, and the standard error was calculated by the equation:

$$\text{standard error} = \text{standard deviation} / \sqrt{\text{Sample Size}}$$

RESULTS

Figure 3 shows the spectrum of a characteristic specimen from RG superimposed on a typical AG specimen. Both spectra reveal the peaks of bands characteristic of the polyurethane structure, such as OH (3380 cm^{-1}), NH (3316 cm^{-1}), aromatic CH (3047 , 1597 , 815 , and

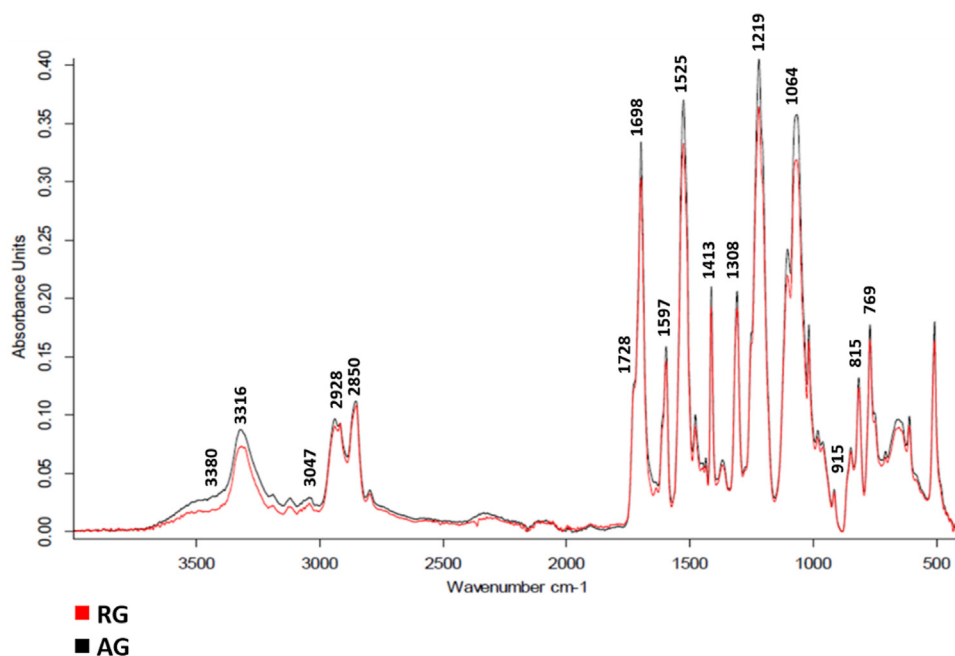


Fig 3. Superimposed ATR-FTIR spectra of RG and AG with the characteristic bands of a polyurethane structure. *ATR-FTIR*, attenuated total reflectance-Fourier transform infrared technique; AG, in vivo aged group; RG, reference group.

769 cm^{-1}), CH (2938, 2850, 1413, and 915 cm^{-1}), C = O (1728 and 1308 cm^{-1}), amide I (C = O of NCO, 1698 cm^{-1}), amide II (NH and C = O of NCO, 1525 cm^{-1}) CO (1219 cm^{-1}) and COC (1100–1067 cm^{-1}). The absorption bands NH (3313 cm^{-1}), amide II (1525 cm^{-1}), and C = O (1738 cm^{-1}) indicate the presence of a urethane structure.²⁹ The similarity between both spectra implies that there are no significant changes in the chemical structure of the material after exposure to the oral environment because the wave numbers of the characteristic bands of the polymer type are maintained, thus denoting the chemical stability of the material.

Figure 4 shows the different intensities for bands NH (3316 cm^{-1}), C = O (1698 cm^{-1}), C = O (1728 cm^{-1}), and C = O (1525 cm^{-1}), respectively. These bands refer to intermolecular hydrogen bond-like interactions that may occur with the hydrogen of the water molecule when there is water absorption by the polymer. There was no displacement in any bands analyzed for higher wave numbers (in the case of NH intermolecular bond) or smaller wave numbers (in the case of C = O intermolecular bond). However, there were slight variations in the intensity of these bands.

Table 1 shows that the ΔV and $W_A\%$ volume values increased gradually. There were no statistically significant differences between mean initial volume and

mean volume at 1 hour ($P = 0.52$), mean initial volume and mean volume at 3 hours ($P = 0.60$), mean volume at 1 hour and mean of volume at 3 hours ($P = 1.00$), and mean volume at 168 hours and mean volume at 336 hours ($P = 0.694$). In relation to ΔV , there were no statistically significant differences between mean ΔV at 1 hour and mean ΔV at 3 hours ($P = 1.00$) and between mean ΔV at 168 hours and mean ΔV at 336 hours ($P = 0.698$). The $W_A\%$ values presented statistically significant differences ($P < 0.05$) among all the time points analyzed.

Figure 5 shows the strong positive correlation ($r = 0.768$; $P < 0.05$) between $W_A\%$ and ΔV over time. It can be observed that the flow of water absorption and volume variation is continuous but variable, increasing expressively after 24 hours and tending toward a certain stagnation at 168 hours. The linear regression equation mathematically explains how the material volume varies depending on the amount of water absorbed and can be expressed as $y = 0.001 + 0.001x$, where x is $W_A\%$ and y is ΔV . The ratio shows that each unit of $W_A\%$ volume varies by 0.002 mm^3 .

Figure 6 shows the color change of some aligners before and after exposure to the oral environment. Observably, they became darker and opaque, although there was a difference in the intensity of these changes.

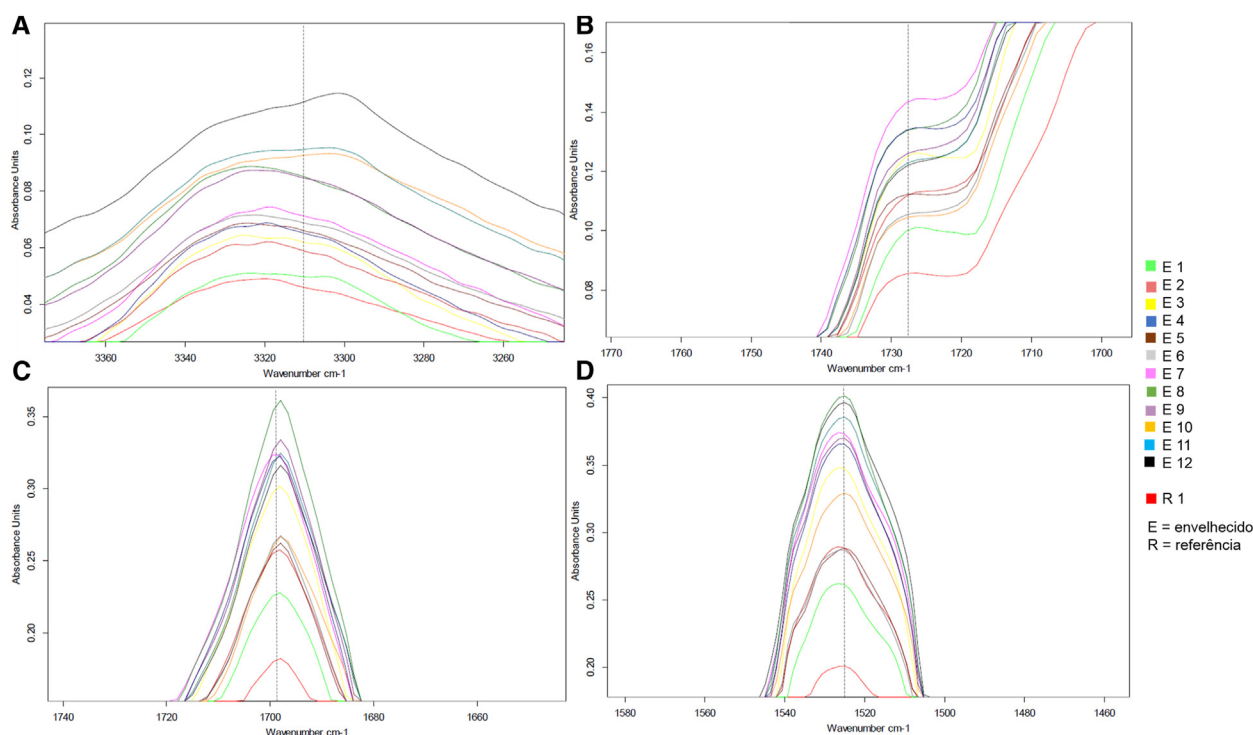


Fig 4. ATR-FTIR Spectra of AG compared with the RG for the different bands: **A**, Band 3316 cm^{-1} ; **B**, Band 1728 cm^{-1} ; **C**, Band 1698 cm^{-1} ; **D**, Band 1525 cm^{-1} . ATR-FTIR, attenuated total reflectance-Fourier transform infrared technique; RG, reference group.

Table II shows the descriptive statistics for parameters L^* , a^* , b^* , and TP. After intraoral exposure, there were statistically significant differences ($P < 0.05$) for L^* , b^* parameters, and TP. In this respect, the L^* and TP values decreased, explaining the dull appearance and greater opacity of the aligners, respectively; the b^* values increased, characterized by their yellowish appearance after use. The mean ΔE value of the aligners was 4.04, later converted to the NBS scale, resulting in a mean value of 3.72 NBS units, which is classified as an appreciable/marked color change. Figure 2 represents the distribution of color change classification according to the NBS scale for all the aligners evaluated in this study; most showed appreciable/marked color change.

Table III presents the descriptive statistics for the elastic modulus and hardness. There was a decrease in the elastic modulus and hardness values, with a statistically significant difference ($P < 0.01$) between RG and AG after exposure to the intraoral environment.

Figure 7 shows the surface of the AG and RG aligners evaluated by optical microscopy and scanning electron microscopy, respectively. Note that the surface of the RG aligners is not regular; it has small cracks. After it was used for 14 days, there seemed to be an increase in

irregularities and faults, such as small distortions and microcracks in the surface of the material, in addition to an incipient formation of biofilm. The EDS analysis shows similar peaks of calcium, phosphate and potassium distribution in AG aligners, not found in RG aligners.

Figure 8 reveals the surface morphology and 3D profile of an RG aligner and an AG aligner. The surface of the AG aligner does not show any homogeneity, nor does the RG aligner (not exposed to the intraoral environment); however, there appears to be increased surface roughness for AG after use of the orthodontic appliance for 14 days, revealing an uneven and heterogeneous surface. Table III shows the descriptive statistics for S_a , indicating no statistically significant difference ($P > 0.05$) between the S_a values of RG and AG.

DISCUSSION

The material constituting the Invisalign aligners was polyurethane, characterized by typical bands that indicate the urethane structure of its composition.²⁹ This result corroborates the findings of other studies.^{5,7,30} In terms of the chemical structure of the aligner, the polymer was found to be stable during the 14 days of use, ascertained by the similarity of the infrared spectra

Table I. Descriptive statistics for volume (mm^3), ΔV , and $W_A\%$ for each period analyzed

Variable	Initial	1 h	3 h	24 h	48 h	72 h	168 h	336 h
Volume (mm^3)								
Mean $\pm$ SD	21.559 $\pm$ 0.001 ^a	21.683 $\pm$ 0.001 ^a	21.767 $\pm$ 0.001 ^a	22.647 $\pm$ 0.002 ^b	23.620 $\pm$ 0.002 ^c	25.133 $\pm$ 0.002 ^d	25.539 $\pm$ 0.002 ^e	25.764 $\pm$ 0.002 ^e
SE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ΔV (mm^3)								
Mean $\pm$ SD		0.124 $\pm$ 0.000 ^a	0.207 $\pm$ 0.000 ^a	1.088 $\pm$ 0.001 ^b	2.061 $\pm$ 0.001 ^c	3.574 $\pm$ 0.002 ^d	3.980 $\pm$ 0.002 ^e	4.205 $\pm$ 0.002 ^e
SE		0.000	0.000	0.000	0.000	0.000	0.000	0.000
$W_A\%$								
Mean $\pm$ SD		0.317 $\pm$ 0.021 ^a	0.357 $\pm$ 0.023 ^b	0.476 $\pm$ 0.031 ^c	1.269 $\pm$ 0.082 ^d	4.441 $\pm$ 0.287 ^e	6.424 $\pm$ 0.414 ^f	6.622 $\pm$ 0.427 ^g
SE		0.005	0.005	0.007	0.018	0.064	0.093	0.096

Note. Different superscripted letters indicate statistical significance among the different time intervals ($P < 0.05$). SD, standard deviation; SE, standard error.

absorbed by the material before and after exposure to the intraoral environment. Regarding the wave numbers and intensities obtained, there was no significant chemical alteration of the material. This result may also help elucidate earlier findings concerning the noncytotoxicity of the aligner material.^{31,32}

In contrast, some reports suggest a chemical alteration of the material after 14 days of use associated with biofilm adsorption.^{7,33} However, it should be noted that the ATR-FTIR technique is extremely sensitive; therefore, the specimens were cleaned beforehand to obtain greater resolving power. This led the authors of the present study to associate the result obtained by this analysis exclusively with the polymer material, thus minimizing interferences regarding the adsorption of any surface biofilm that could mask the real spectrum of the material. However, the subtle increase in peak intensity between the 4000–3300 cm^{-1} regions may be related to hydrogen bonds in the -OH and -NH groups of proteins and carbohydrates. These could be related to forming an irregular proteinaceous biofilm, thus corroborating the findings of a previous report.³⁴ However, this formation does not appear to cause chemical changes on the surface of the polymer. As demonstrated, the polymer absorbs water after exposure to the intraoral environment, and, according to a previous report,^{31,35} the hydrogen of the water molecule may interact with the polyurethane structure, forming hydrogen bonds between 2 C = O groups, occupying the hydrogen bonding sites in the NH group, or remaining in their free form. Any of these forms of interaction may promote changes in the mechanical and physical properties of the polymers.^{33,34} The occurrence of a discrete difference in the absorption intensity of specific bands (3316, 1728, 1698, and 1525 cm^{-1}) may be related to the water absorption observed in the polymer and consequently to the possible formation of hydrogen bonds between the NH and C = O groups.³³ Nevertheless, this molecular interaction did not result in the formation of by-products or representative chemical alterations characterized by the conversion or disappearance of these bands. This finding is similar to that of a previous study,⁵ and makes us believe that most of the water absorbed by the aligners might be in the free form. The relatively short time the material is exposed to the oral environment may contribute to the lack of relevant chemical changes in the polymer. The diffusion of the water in the organic matrix can cause hygroscopic expansion, resulting in an increase in volume and/or weight. This consequence may promote changes in the mechanical and physical properties of the material.^{15,16,36}

This study revealed that polyurethane gradually absorbs water over 2 weeks on exposure to artificial saliva.

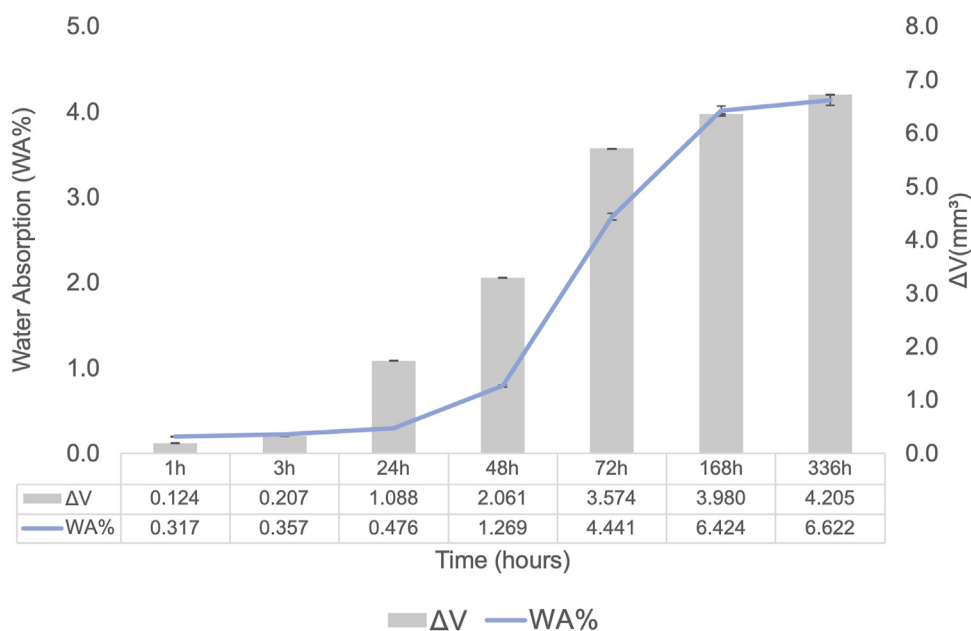


Fig 5. Correlation between $W_A\%$ and ΔV in 336 hours (2 weeks) of immersion in artificial saliva. The error bars are calculated based on the standard error of ΔV and $W_A\%$.

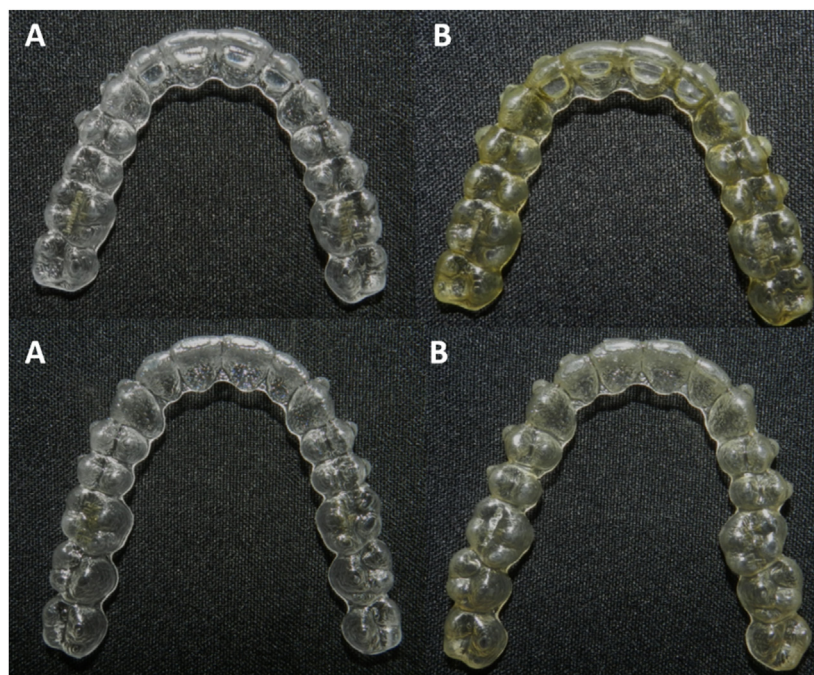


Fig 6. Photographs of orthodontic aligners: **A**, Before exposure to the intraoral environment; **B**, After exposure to the intraoral environment for 14 days.

A similar previous study¹³ indicated that aligners composed of polyurethane had a higher water absorption rate when immersed in distilled water than aligners

fabricated using polyethylene terephthalate glycol and polycarbonate. This study found a strong, positive correlation among the variables of water absorption rate and

Table II. Mean, standard deviation and *P* value for color parameters: *L**, *a**, *b**, and TP

Group	<i>L</i> *	<i>a</i> *	<i>b</i> *	TP
RG	89.764 ± 1.108	0.534 ± 0.116	4.076 ± 0.312	48.977 ± 0.825
AG	86.632 ± 2.465	0.674 ± 0.320	6.623 ± 1.624	46.362 ± 1.895
<i>P</i> value	0.001*	0.177	<0.001*	0.001*

Note. Values are presented as mean ± standard deviation.

AG, in vivo aged group; RG, reference group.

*Statistically significant difference.

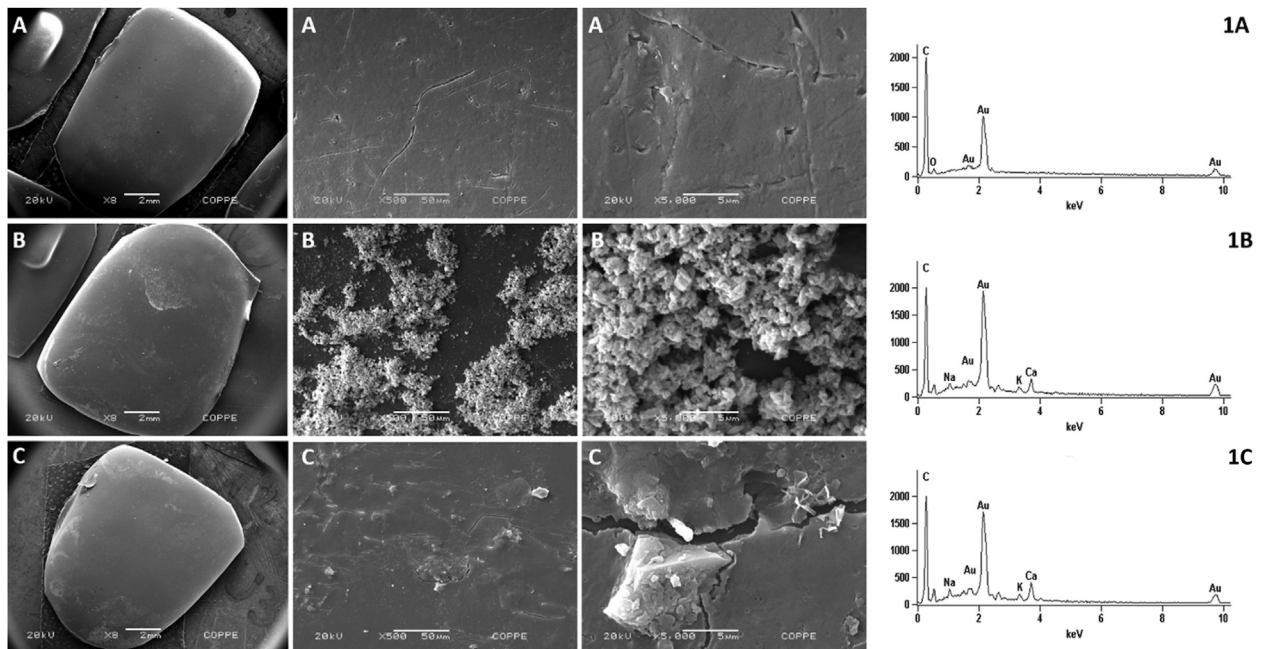


Fig 7. SEM examinations and spectra EDS of the buccal surface of orthodontic aligners: **A**, RG aligner at magnifications of 8×, 500×, and 5000×, respectively; **B** and **C**, AG aligners at magnifications of 8×, 500×, and 5000×, respectively. **1A**, spectra EDS of an RG aligner at region A at 5000× magnification; **1B**, spectra EDS of an AG aligner at region B at 5000× magnification; **1C**, spectra EDS of an AG aligner at region C at 5000× magnification. SEM, scanning electron microscope; EDS, energy dispersive spectroscopy; AG, in vivo aged group; RG, reference group.

volume variation. The water sorption process in the polymers is considered multifactorial and may occur largely because of the hydrophilic nature of its polymer units, its amorphous and/or crystalline structure, and its intermolecular spaces and porosities.^{15,37} Thermo-plastic polyurethanes have both a hard segment and a soft segment. This particularity might influence their water absorption capability because this property is usually connected to the amount of free volume available in the structure of the material.^{38,39} In addition, polyurethanes are characterized by urethane hydrophilic units (-NHCOO-), which can form hydrogen bonds with water, thus facilitating their absorption.⁴⁰ This study had statistically significant absorption rates when comparing

the time interval of 1 and 3 hours; however, when comparing the time intervals of 168 and 336 hours, there was no difference in the absorption rates, despite the volume variation (Table I). Therefore, as pointed out, the material begins to undergo dimensional changes only after 24 hours of immersion in artificial saliva, and the changes in volume between 168 and 336 hours are statistically insignificant. Accordingly, the material appears to attain some dimensional stability after 168 hours (1 week) of use of the aligner. This phenomenon can be explained by the linear equation obtained from the regression analysis of the model, namely that a certain absorption rate is required for the volume variation to be noticed more significantly. The importance of

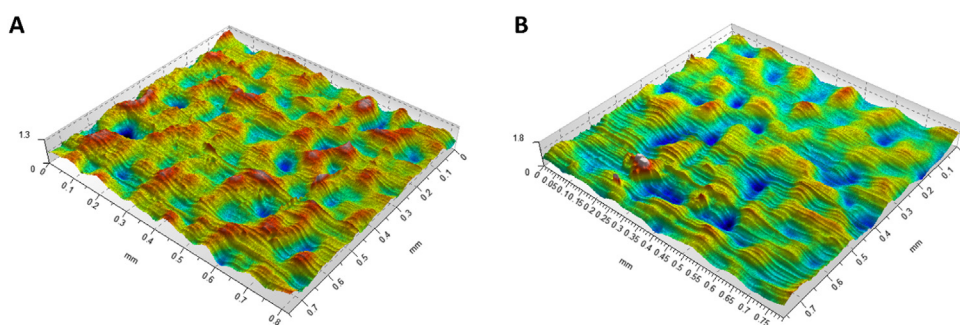


Fig 8. Three-dimensional photograph of the morphologic surface of orthodontic aligners: **A**, RG aligner; **B**, AG aligner. *Red*, peaks; *Blue*, valleys. AG, in vivo aged group; RG, reference group.

Table III. Mean, standard deviation, and *P* value for the *E*, *H*, and *S_a*

Group	<i>E</i> (MPa)	<i>H</i> (MPa)	<i>S_a</i> (μm)
RG	640.75 ± 4.73	36.25 ± 1.05	4.815 ± 2.452
AG	405.16 ± 6.07	26.50 ± 0.8	6.785 ± 2.248
<i>P</i> value	<0.01*	<0.01*	0.052

Note. Values are presented as mean ± standard deviation.

AG, in vivo aged group; RG, reference group.

*Statistically significant difference.

the study of dimensional changes resulting from the hygroscopic expansion of polymeric materials is asserted when considering that this alteration may affect the adaptation of orthodontic aligners composed of polyurethane, such as Invisalign, resulting in changes in the orthodontic forces transmitted to the tooth.^{13,41,42} In addition, dimensional changes may impair the outcome of orthodontic treatment because each aligner is designed to promote a 0.25–0.30 mm movement of a tooth or a small group of teeth^{43,44} after every 14 days. This means that dimensional changes may result in a reduced amount of tooth movement that may impair how well the aligner moves because a change in the aligner size implies that a portion of the space designated to move the tooth will be occupied by the aligner.

Discoloration of the material occurs after 14 days of intraoral exposure and mainly affects the parameters of luminance *L** and the *b** scale (yellow to blue), making it significantly darker and yellowish. The color change undergone by the aligners may be associated with the adsorption or penetration of pigments into the polymer, derived from the diet of each individual (the oral flora), temperature variation, the form and frequency of hygiene, the pigments and characteristics of the saliva (enzymes and pH), and the quality of the surface of the material.^{20,22,24,45} Some studies^{22,24,45} have

demonstrated that polyurethane-based materials are vulnerable to the absorption of pigments and generally do not promote adequate color stability. In addition, the results obtained in this study for the absorption rates of artificial saliva also help elucidate the ease of pigmentation of the material because the water molecules bind and penetrate the surface of the material, thus facilitating the adsorption and penetration of pigments.¹³ The presence of -NHCOO- in polyurethane-based polymers such as Invisalign^{5,7,30,46} makes them color-unstable because this polarity interacts with the hydrophilic pigments in saliva and liquids,⁴⁰ thus facilitating discoloration and decreased translucency, as was observed. During the exposure to the intraoral environment, the aligners showed microcracks and porosity, which may facilitate greater penetration of pigments, and a consequent increase in discoloration, as evaluated by SEM analysis.^{24,47} The increase in the roughness of the material may also promote increased pigmentation and loss of transparency.^{24,46} From a clinical point of view, the color changes presented by the aligners in the present study are considerable and appreciable according to the NBS scale. This color instability can affect social perception, which must be borne in mind because the aligner attempts to present itself as an extremely esthetic orthodontic apparatus. Therefore, the color stability of the material should offer a desirable proposition.²² Turning the surface of aligners into a more hydrophobic polyurethane by modifying the polymer with silicone or fluorinated compounds is an option adopted to achieve greater color stability and may soon be a reality for orthodontic aligners. At the same time, research is underway to promote the blending of polymers by diversifying and optimizing their properties, especially mechanical properties.^{41,42,48}

In general, specimens of specific sizes were required to evaluate the mechanical properties of the materials.^{13,49} These technical limitations were duly offset

by using the nanoindentation technique and by the method proposed by Oliver and Pharr.^{25,26} Based on the results of their study, the elastic modulus and polymer hardness were significantly affected after 14 days of orthodontic aligner exposure to the oral environment. This result corroborates a previous clinical study using the same type of polymer.⁵ From a mechanical point of view, the decrease in the elastic modulus values implies greater deformation of the material with the same applied load, attenuating the force supplied by the material to promote dental movement.⁵⁰ However, this attenuation ultimately brings about the intended treatment, especially when there is a degradation of the mechanical properties and alteration of the material dimensions (volume), as found in this study. In addition, we found that the modulus of elasticity was about 63% lower between RG and AG; this leads us to deduce that after 14 days of clinical use resulting in the same deformation, we would have 63% lower tension. There was also a significant decrease in the hardness values of the material. The magnitude of the decrease can be attributed to the wear resistance of the material.³⁰ This deterioration of the mechanical properties of the polymer can be explained by the softening mechanism of the polyurethane. Thermoplastic polymers are characterized by a 2-phase microstructure composed of rigid and flexible segments. Soft segments create disordered amorphous regions, whereas rigid segments make up polar molecules that form hydrogen bonds resulting in regions with more well-ordered orientations. The softening mechanism has been associated with a perpendicular orientation of the rigid segments to the applied stress in the cases of deformation, with the fragmentation of these regions into smaller portions that promote greater deformation.^{34,41} The ATR-FTIR analysis showed a small variation in the intensities of the C = O bonds (1728, 1698, and 1525 cm^{-1}), which may explain this phenomenon.⁵ From a clinical point of view, the results of this study indicate that orthodontic forces are exerted by polymer decay during orthodontic treatment, corroborated by previous studies.^{5,50-52} In addition, there is a certain consensus among studies^{50,52} indicating that most of the orthodontic force exerted by some aligners occurs during the first 24 hours of use, reaching a plateau, after which the level remains the same until the end of 14 days of use. There are indications that Align Technology may change its clinical aligner use protocol to weekly rather than fortnightly exchanges, depending on the specifics of each patient and mechanics. This study provides initial information for system optimization. However, more clinical studies are needed to enable careful

evaluation of the material initially and in intermediate and subsequent stages during treatment, to ultimately allow this practice to be established in the orthodontic clinic, especially taking into account the biology of tooth movement.^{5,50}

The microscopic findings of this study revealed that the surface morphology of the aligners is irregular, even those never exposed to the intraoral environment. This may be attributed to the lack of control in the manufacturing process of these aligners. However, based on this study, these irregularities tend to be more pronounced as the material becomes more exposed to the adversities of the intraoral environment, either because of the increase in porosities or to the development of cracks and small distortions.⁴⁶ These factors may contribute to increasing the surface roughness of the material and consequently be conducive to biofilm deposition.⁵³ Enzymatic action, pH changes, consumption of acidic beverages, the potential abrasive effect of masticatory movements and tooth brushing may exacerbate the irregularities and heterogeneity of the surface, leading to increased surface roughness.⁴⁶ Although the surface roughness of the material increased because of exposure to the oral environment, the mean values between the 2 groups were not statistically significant. Possibly, the 14-day oral exposure time was not sufficient for these changes to be significant. Based on the results obtained by the distribution of calcium, potassium, and sodium on the surface of the aligners exposed to the oral environment, it may be concluded that the biofilm was in the process of calcification, similar to the findings of previous studies.^{7,46}

Therefore, after 14 days of exposure to the oral environment, the transparent orthodontic aligners remained chemically stable; however, they underwent dimensional change resulting from gradual water absorption, degradation of mechanical properties, a color change that became darker, yellowish and opaque, and irregularities in surface morphology.

CONCLUSIONS

Exposure of the constituent polymer of the transparent Invisalign aligners to the intraoral environment for 14 days revealed that:

1. The aligners are made of thermoplastic polyurethane and have proven chemically stable.
2. There is a gradual increase over time in the rate of water absorption and a variation in the volume of the polymer, showing a strong correlation between these variables.

3. The orthodontic aligners presented significant discoloration and loss of transparency, becoming darker, yellowish and opaque.
4. The mechanical properties of the material were significantly reduced.
5. The surface roughness of the material tended to increase, and modifications occurred in the morphology and surface topography of the aligners, characterized by the absorption of biofilm and the appearance of microcracks, grooves, and distortions.

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AUTHOR CREDIT STATEMENT

Larine Ferreira Lira contributed to data curation, formal analysis, investigation, and original draft preparation; Eduardo Otero Amaral Vargas contributed to manuscript review and editing and validation; Eduardo Moreira da Silva: methodology, supervision, and validation; Juliana Nunes da Silva Meirelles Dória Maia contributed to methodology, software, and supervision; Amal Elzubair contributed to methodology, software, and supervision; Liliane Siqueira de Morais contributed to project administration and funding acquisition; Sérgio Alvaro de Souza Camargo, Jr contributed to formal analysis and manuscript review and editing; Glaucio Serra contributed to project administration and manuscript review and editing; and Margareth Maria Gomes de Souza contributed to project administration and manuscript review and editing.

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