

Short-term chemical and physical changes in Invisalign appliances

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Objective: To investigate the short-term optical, chemical and morphological changes in Invisalign appliances.

Methods: One 'as-received' Invisalign aligner, one 'as-received' Invisalign aligner immersed in artificial saliva for 14 days and 10 Invisalign aligners worn by 10 randomly selected patients for 14 days were used. Fourier transform infra-red microspectroscopy was used to characterise any molecular changes on the surfaces of the appliances, spectrophotometry was used to evaluate any changes in colour and transparency, scanning electron microscopy and energy dispersive X-ray microanalysis were used to examine the surface morphology and elemental composition of deposits on the surfaces of the aligners and gas chromatography-mass spectrometry was used to identify substances released from the aligners into the artificial saliva.

Results: Aligners worn for 14 days had microcracks, abraded and delaminated areas, localised calcified biofilm deposits and loss of transparency. Monomers and/or by-products were not released from the aligner suspended in artificial saliva for 14 days, which suggests that the material is chemically stable.

Conclusions: Further studies are required to evaluate how intra-oral conditions may influence the optical properties and chemical stability of the aligners.

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Introduction

Many adult orthodontic patients with mild malocclusions request appliances that are 'invisible', especially during smiling. These requests have led to the development and refinement of lingual appliances and sequential, transparent aligners.^{1,2} Aligners are produced via a thermoforming process on accurate models of patients' malocclusions.³ Despite the high level of precision during manufacture, they do not maintain their original shape or composition in the mouth. Over time they slowly degrade and loosen as teeth are moved into position and the aligner changes shape. Although polyurethane, the constituent of the

aligners, is biocompatible, it is not an inert material and is sensitive to heat, humidity and prolonged contact with salivary enzymes.⁴

Aligners and some retainers should be worn continuously, except during eating and oral hygiene procedures. As a rule, aligners are replaced every 14 days during treatment and, thus, each aligner is in the mouth for approximately 308 hours. During this period the aligner is continually exposed to salivary enzymes, introduced liquids, inhaled and exhaled air and trauma caused by swallowing, speech and bruxism. There have been few studies reporting the deterioration of the aligners during use due to the

difficulty in monitoring intra-oral conditions and aligner wear. Schuster et al.⁴ investigated the effects of exposure to the oral environment on Invisalign aligners and reported substantial alterations in their morphology, but not in their composition, over two weeks.

The aim of the present study was to determine the short-term optical, chemical and morphological changes in Invisalign appliances.

Materials and methods

The following materials were used: one 'as-received' Invisalign aligner (N); one 'as-received' Invisalign aligner immersed in a container filled with artificial saliva for 14 days (A); 10 Invisalign aligners worn by 10 randomly selected patients for 14 days (U). The optical, chemical and morphological characteristics of the aligners were investigated with Fourier transformation infra-red spectroscopy (FT-IR), scanning electron microscopy equipped with an energy-dispersive X-ray probe for microanalysis (SEM-EDX), spectrophotometry (SP) and gas chromatography-mass spectrometry (GC-MS).

To simulate intra-oral aging, the 'as-received' aligner was immersed in Oral Balance (Biotène Oral Balance, Biopharm Sas, Peschiera Borromeo, Italy), a saliva substitute used by patients with xerostomia. This saliva substitute is composed of purified water, hydrogenated starch hydrosylate, xilitol, hydroxy-ethyl cellulose, glycerate polymethacrylate, beta-d-glucose, lactoperoxidase, lysozyme, lactoferrin, glucose oxidase, potassium, thiocyanate and aloe vera. The aligner was immersed in 50 ml of artificial saliva, and placed in a muffle furnace at a constant temperature of 37°C for 22 hours per day for 14 days. The aligner was removed from the artificial saliva solution twice a day, cleaned with a toothbrush under running water to remove the gelatinous residue and dried with compressed air. It was stored in its case until one hour had elapsed and then returned to the muffle furnace. This procedure simulated removal of the aligner from the mouth at mealtimes.

Ten randomly selected patients, wearing aligners as part of their orthodontic treatment, were asked to wear their aligners 22 hours per day. The aligners were removed from the mouth twice a day, cleaned with a toothbrush under running water and immersed in a 2 per cent solution of the water-based disinfectant Amuchina (Gruppo Angelini, Italy) for

15 minutes. The concentration of the disinfectant was that recommended by the manufacturer. After 15 minutes the aligners were removed, washed under running water, stored in their cases for 45 minutes and then returned to the patients. Patient compliance with the suggested protocol was not verified.

At the end of the 2-week experimental period, the optical, chemical and morphological changes in the as-received (N), artificially aged (A) and intra-orally aged aligners (U) were investigated.

Fourier transformation – infra-red analysis (FT-IR)

Molecular changes on the surfaces of the aligners were examined with Fourier transformation – infra-red analysis. Spectral transmittance data were obtained in the mid infra-red frequency range using a Perkin-Elmer Spectrum GX FT-IR spectrometer equipped with a Perkin-Elmer Autoimage microscope. The spectral resolution was 4 cm⁻¹ and the spatial resolution was 100 × 100 mm. The spectra were the result of 256 scans. For readings in the near infra-red frequency range (NIR), a Perkin Elmer Spectrum One NTS spectrometer was used. The spectral resolution of the latter instrument was 16 cm⁻¹ and the spectra were the result of 256 scans. Each aligner was cut into rectangular fragments with surface areas of approximately 0.5–1 cm² and examined with FT-IR. Baseline (polynomial line fit) was performed in all cases while second derivative Fourier self-deconvolution and curve fitting (Gaussian character) procedures were used to isolate the bands of interest. The bands were assigned using previously published data.⁸ The data were examined with Spectrum v.303 (Perkin-Elmer, Monza, Italy) and Grams AI (Thermo Galactic, Salem, NH, United States) software packages. Although the majority of the spectral information can be registered in the mid infra-red frequency range, unresolved overlapping absorptions may be present in this frequency range. In such a case the near infra-red combination bands can be useful sources of chemical information.

Scanning electron microscopic – energy dispersive X-ray analysis (SEM-EDX)

Scanning electron microscopy – energy dispersive X-ray analysis was used to identify the composition of substances deposited on the surface of each aligner. Samples, approximately 5 × 5 mm, were cut from the

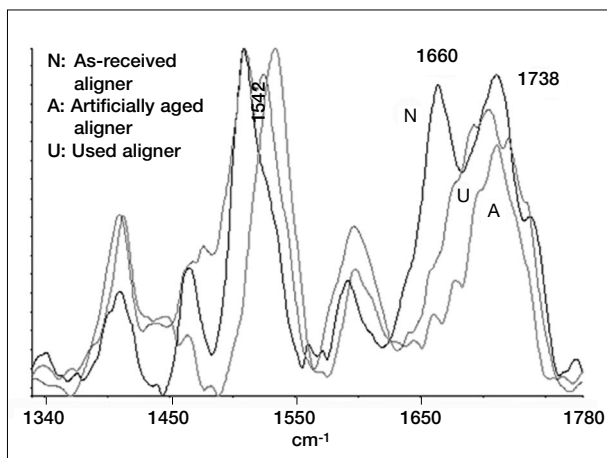


Figure 1. FTIR spectra of the as-received (N), artificially aged (A) and intra-orally aged (U) aligners in the region 1300–1800 cm^{-1} .

central incisor, canine, first premolar and third molar areas (Teeth: 11, 13, 14 and 18) of each aligner, sputter-coated with gold and carbon and examined under a scanning electron microscope (SEM) equipped with an energy dispersive X-ray micro-analysis system (EDX).

Spectrophotometric analysis (SP)

A Varian Cary UV spectrophotometer was used to analyse the changes in colour and transparency of each aligner. The spectra were registered in transmission mode against air in the visible spectrum (Band frequency: 900–300 nm).

Gas chromatography-mass spectrometry analysis (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) was used to determine if elements leached from the aligner suspended in artificial saliva over 14 days. Ten ml of fresh Oral Balance, used as the reference sample, and 10 ml of Oral Balance from the immersion solution were analysed with a gas chromatography – mass spectrometer equipped with an electron impact ionisation detector. Approximately 10 ml of the artificial saliva was extracted with 10 ml high performance liquid chromatography (HPLC) grade dichloromethane, and the extract dried over anhydrous sodium sulfate. A Supelco SPB-5 column of 30 m in length, ID (internal diameter) 0.2 mm and 0.25 mm film thickness, was used with the carrier gas at a

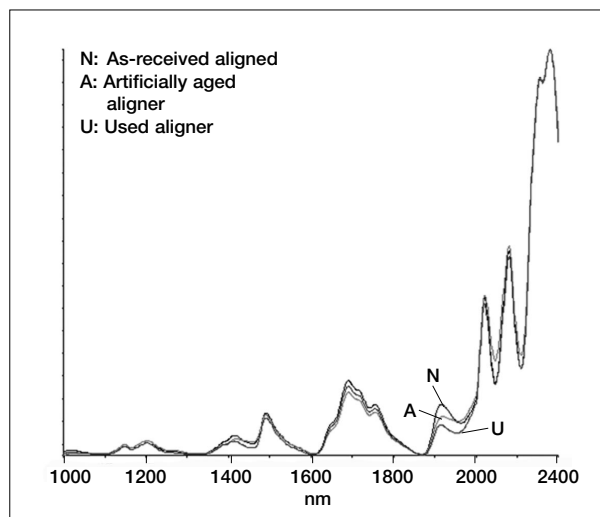


Figure 2. FTIR spectra of the as-received (N), artificially aged (A) and intra-orally aged (U) aligners in the near infrared region.

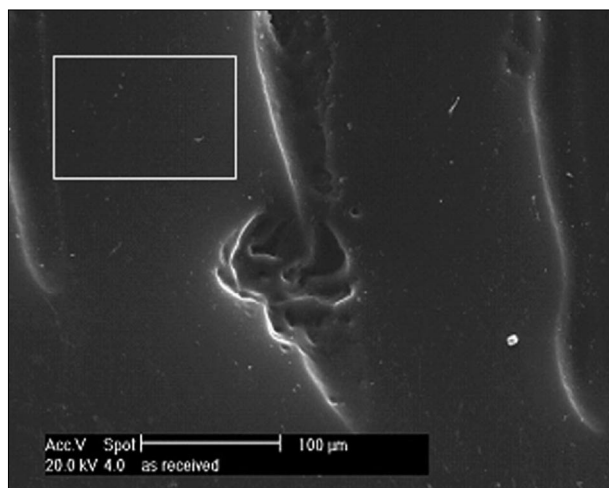
flow rate of 30 cm/msec. The column programme was 5 minutes at 40°C, 140°C (inter-mediate temperature) at a rate of 5°C per minute, 290°C (final temperature) at 10°C per minute and 20 minutes holding time.

Results

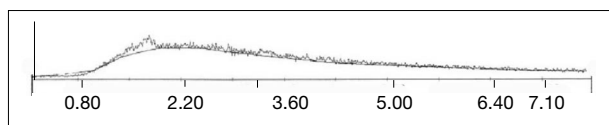
Spectroscopic determinations in the mid infra-red region

Figure 1 shows representative spectra of the as-received (N), artificially aged (A) and intra-orally aged (U) aligners after 14 days. Major changes are evident in all specimens in the spectral region 1300 to 1800 cm^{-1} , indicating that molecular changes occurred on the surfaces of all the aligners. When the spectral profiles of the aligners were compared, the following points emerged:

1. In the N specimen, the bands at 1660 and 1738 cm^{-1} correspond to the following components: free and hydrogen-bonded carbonyl ($\text{C}=\text{O}$) as found in urethane. The typical convoluted pattern of H-bonded carbonyl is shown by A and a pattern with disordered hydrogen-bond conformations in U.⁹
2. The 'intensity' (i.e. the height of the peaks) and 'stretching' of the bases (i.e. the width across the bases) of the carbon-carbon double bond ($\text{vC}=\text{C}$) vibrational mode increased with the aging of the aligner (U with respect to N). This feature, together with the broadening of carbonyl ($\text{C}=\text{O}$) vibration,



(a)



(b)

Figure 3. (a) SEM micrograph of the as-received aligner used as a reference.

(b) EDX spectrum of the area enclosed by the rectangle in (a).

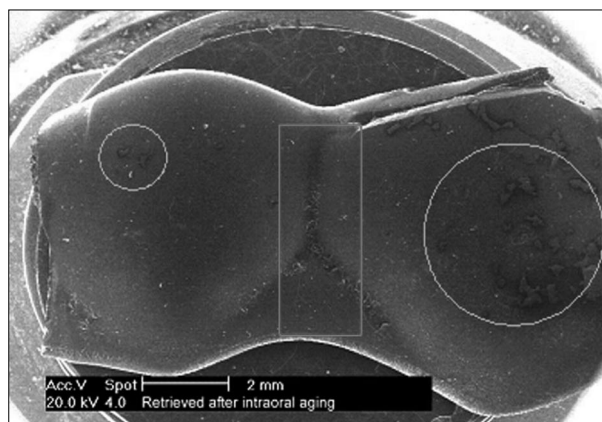
can be attributed to formation of a carbon coating with a number of unsaturated carbon-carbon double bonds ($C=C$).¹⁰

3. The broader base in U between 1500 and 1560 cm^{-1} , as compared with N and A, indicates symmetrical deformation of the C-H bond and aromatic skeletal vibrations.¹¹

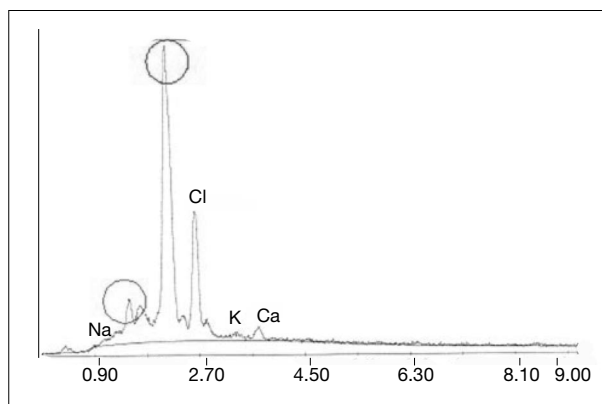
4. Changes in shape and intensity of the bands at 1355 cm^{-1} , 1420 cm^{-1} and 1470 cm^{-1} which together with the band at 1610 cm^{-1} could be due to a decrease in the isocyanate group, following hydrolysis reaction by the ambient medium.¹⁰

Spectroscopic determinations in the near infra-red region (NIR)

NIR spectra of polyurethanes showed carbonyl ($C=O$) and amine (N-H) groups (overlapping bands) at 1900–2100 cm^{-1} , 1350–1350 (N-H) and 1100–1250, 1600–1800 and 2100–2450 ($C-H$ bond) nm .¹² Nevertheless, some spectral differences between the specimens can be seen in Figure 2. For



(a)



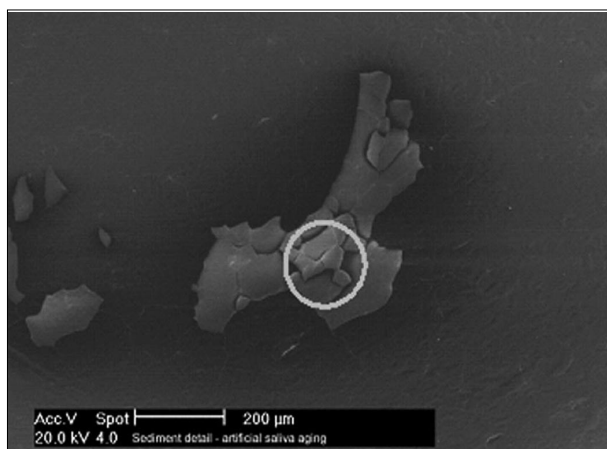
(b)

Figure 4. (a) SEM micrograph of an aligner after intra-oral use. (b) EDX spectrum of the area enclosed by the larger circle in (a). The high peak (circled) is due to the gold sputter coat and the low peak (circled) is due to the aluminum in the stub holding the specimen.

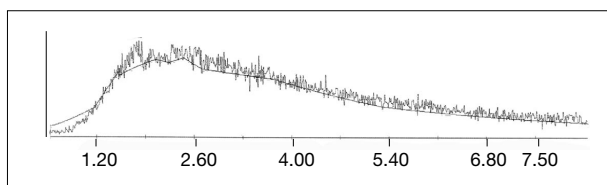
example, the spectra of the intra-orally aged aligners (U) differ from those found in the as-received aligner (N): the observed peaks around 1500, 1700 and 1900 nm , due to changes in $C=O$ and N-H absorption, are shorter. Minor changes in these regions are also present in the spectra of the A aligners, showing an intermediate behaviour between N and U. These findings are in agreement with those in the mid infra-red region indicating that the artificially aged samples (A) also underwent some chemical modification.

Scanning electron microscopy (SEM-EDX)

Scanning electron microscopy and energy dispersive X-ray analysis (SEM-EDX) revealed that there was no surface damage or detectable loss of elements from the as-received (N) aligner (Figure 3). In the aligners



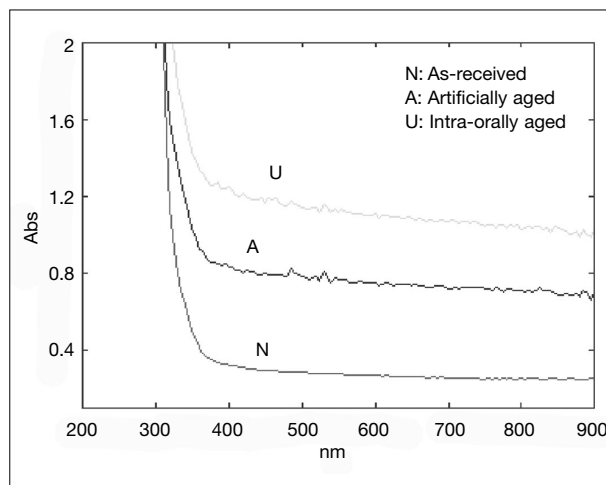
(a)



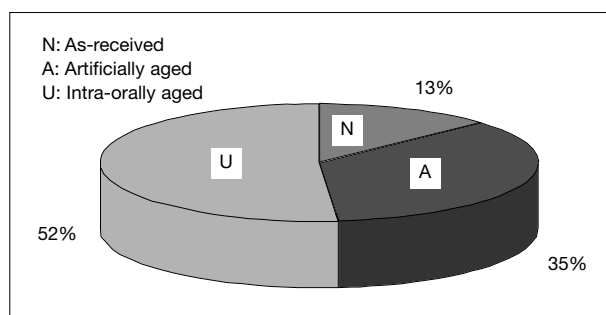
(b)

Figure 5. (a) SEM micrograph of sediment on an artificially aged aligner. (b) EDX spectrum of the area enclosed by the circle in (a).

worn by patients (U) significant damage occurred (Figure 4). The surface of the aligner in the interproximal area showed delamination and areas overlying the occlusal surfaces had amorphous calcified deposits (Figure 4). In the region defined by the rectangle a delamination phenomenon is visible. The circles highlight areas with amorphous, calcified deposits as confirmed by the EDX spectrum (Figure 4b). Chlorine ions on the aligner were attributed to the disinfectant solution used to clean the aligners. The high peak in Figure 4b was due to the presence of gold from the sputter coating and aluminium ions were due to the aluminium stub used to hold the sample during scanning. The analysis was repeated using carbon-coated specimens and no gold or aluminium were found on the surface of the aligner. EDX microanalyses confirmed the presence of calcium, sodium and potassium ions on the surfaces of the aligners retrieved from the patients (U). Similar results were obtained from the incisor, canine, first premolar and third molar areas on the aligners that had been worn for 14 days. No detectable signs of distortion or cracking were found on the artificially



(a)



(b)

Figure 6. (a) Absorbance versus wave length, in the visible range of frequencies. As-received aligner (N), artificially aged (A) and the mean value for 10 intra-orally aged (U) aligners.

(b) Relative opacity of the aligners, in per cent. As-received (N), artificially aged (A) and intra-orally aged (U).

aged aligner (A), but some amorphous surface deposits could be seen on the micrographs (Figure 5). These deposits were probably from the constituent(s) of the Oral Balance solution. EDX microanalysis confirmed that no calcium, sodium and potassium ions were present.

SP analysis

The as-received (N) aligner was more transparent than the artificially aged (A) aligner or the intra-orally aged (U) aligners (Figure 6a). The relative opacities of the aligners are shown in Figure 6b.

GC-MS results

A gas chromatography-mass spectrometer equipped with an electron impact ionisation detector (GC-MS)

revealed that no substances were released from the aligner (A) into artificial saliva over 14 days.

Discussion

In the present study the short-term optical, chemical and morphological changes in Invisalign appliances were determined after 14 days wear. The aligners showed micro-cracks, abrasions, delamination, localised calcified biofilm deposits and loss of transparency. Delamination or separation of the polyurethane material into mica-like layers can lead to significant loss of mechanical strength, and loss of transparency is a serious disadvantage from the patient's perspective. We found no evidence that monomers or by-products were released from the aligner suspended in artificial saliva for 14 days, which suggests that the material is chemically stable. Further intra-oral studies are required, however, to substantiate this suggestion and to determine how intra-oral conditions influence the optical properties of aligners. Our results agree with previous studies that a wide array of 'aging' phenomena occur in aligners retrieved from patients.^{4–13}

We used Fourier transformation – infra-red analysis to demonstrate that molecular changes occurred on the surfaces of the aligners retrieved from patients. FT-IR is a failure analysis technique that provides information about the chemical bonding or molecular structure of materials, whether organic or inorganic. The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infra-red rays absorbs infra-red energy at frequencies which are characteristic to that molecule. We found evidence of molecular changes on the surfaces of the aligners, but our methods did not allow us to determine if these surface changes resulted in material being released into the saliva.

We suspended unused aligners in artificial saliva for 14 days and confirmed with GC-MS analysis of the solvent extracts that the aligners were chemically stable. However, our immersion test may have underestimated the chemical stability of the material as the aligners were not subjected to the hostile conditions present in the mouth.^{14–15} Even during short-term intra-oral use, the aligners were subjected to a variety of chemical, physical and microbial challenges that may encourage the release of by-products and particles from the aligners into the mouth. Future in vivo

studies should investigate this aspect over an extended period of time as retainers and splints are made from the same material and are worn for much longer periods of time.

Major molecular changes were found on the surfaces of the intra-orally aged aligners as compared with the artificially aged aligners. FT-IR analysis revealed different spectral profiles that we have attributed to the presence of morphologically-disordered structures, corresponding to micro-cracks in the interproximal areas of the intra-orally aged aligners, as a consequence of partial fragmentation of polymer chains. The reduced number of isocyanate chemical groups, as suggested by the changes in the spectral range 1470–1350 cm⁻¹ of the mid infra-red region, was confirmed by near infra-red findings.

SEM-EDX analyses of samples taken from the intra-orally aged group showed significant morphological changes. These changes which included cracking, wear at contact points with the opposing teeth, delamination and calcified deposits on the surfaces of the aligners, particularly in stagnation areas such as central fissures, resulted in aligners that were noticeably more opaque than the unused aligner. Trauma derived from chewing and bruxism almost certainly contributed to the opacity of the intra-orally used aligners. Our X-ray microanalyses of selected regions of the used aligners demonstrated an almost uniform distribution of potassium, sodium, chlorine and calcium ions consistent with the initiation of the biofilm calcification process.

Conclusions

Invisalign aligners retrieved from patients after 14 days wear displayed optical, chemical and morphological changes compared to new specimens. Monomers and/or by-products were not released from the aligners suspended in artificial saliva. The retrieved aligners were noticeably more opaque than the as-received and artificially aged aligners. Further studies are required to evaluate how intra-oral conditions may influence the optical properties and chemical stability of aligners.

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