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Structural Conformation and Leaching From In Vitro Aged and Retrieved Invisalign Appliances*

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Abstract

The objectives of this study were to investigate the structure of Invisalign appliances (Align Technology, Santa Clara, Calif) after intraoral exposure, and to qualitatively and quantitatively characterize the substances leached from the aligners after accelerated in vitro aging. Samples of Invisalign appliances were randomly selected from 10 patients before intraoral placement and after retrieval, and the prepared specimens were subjected to (1) bright-field optical reflection microscopy to study the surface morphology; (2) Fourier transform infrared microspectroscopy to characterize the in vivo changes in molecular composition induced on appliance surfaces, (3) scanning electron microscopy and energy dispersive X-ray microanalysis to identify the elemental composition of integuments formed on the surface, and (4) Vickers hardness (HV_{200}) testing. Another set of reference and retrieved appliances was subjected to artificial aging for 2 weeks, and the extracts were subjected to gas chromatography-mass spectroscopy. The retrieved appliances demonstrated substantial morphological variation relative to the as-received specimens involving abrasion at the cusp tips, adsorption of integuments, and localized calcification of the precipitated biofilm at stagnation sites. Buccal segments of retrieved appliances showed an increase in hardness, which might be attributed to mastication-induced cold work; however, the clinical implication of this effect on mechanotherapy is unknown. In vitro aged and retrieved appliances were found to leach no traceable amount of substances in an ethanol aging solution.

Contemporary orthodontics has witnessed the introduction of clear polymeric aligners—Invisalign appliances (AlignTechnology, Santa Clara, Calif)—as a potential alternative to conventional brackets and archwires.[1, 2] Patients are instructed to wear each set of aligners for a minimum of 2 weeks, for 22-hours per day, to achieve gradual tooth movement.[2] Although some disagreement exists over the efficiency and limitations of this method, no study has recorded the aging pattern of the appliances in vivo; this might be pivotal in determining the biocompatibility of the technique.

Polyurethane, the basic constituent polymeric component of Invisalign aligners, is not an inert material and is affected by heat, moisture, and prolonged contact with enzymes.[3, 4] However, no information is available on possible structural alterations of aligners after intraoral exposure. Because monitoring the exact mechanisms involved in aging of materials intarorally is impossible, simulating this process involves artificial in vitro environments to induce an accelerated aging process.[5]

The hypothesis tested in this study was that exposing the appliances to the intraoral environment adversely affects their surface morphology and composition. Thus, the objectives were 2-fold: to investigate the structure of appliances before and after intraoral exposure, and to qualitatively and quantitatively characterize the substances leached from the aligners after accelerated in vitro aging.

Material and methods

Samples of Invisalign appliances were randomly selected from 10 patients. The samples included reference aligners collected before intraoral placement and 12 samples taken from aligners worn intraorally for 2 weeks, approximately 22 hours per day. Specimens of the latter group included 4 aligners from visibly intact areas, 4 specimens from visibly worn areas, and 4 samples macroscopically demonstrating calcified regions.

Characterization of retrieved specimens

All specimens were prepared by carefully cutting samples approximately 5 X 5 mm under a stereomicroscope from buccal segments, cuspal areas, and central fissures of molar regions. All samples were then subjected to:

- Bright-field optical reflection microscopy with a microscope (Eclipse ME 600, Nikon, Kogaku, Tokyo, Japan) equipped with linear polarizers to study surface morphology and to identify birefringent regions of the reference and intarorally exposed specimen groups.
- Fourier transform infrared microspectroscopy (FTIR) to characterize the in vivo changes in molecular composition induced on appliance surfaces. Spectra acquisitions were performed on an FTIR spectrometer (Spectrum GX, Perkin-Elmer Corp, Norwalk, Conn) equipped with an FTIR microscope (Multiscope, Perkin-Elmer) operated under the following conditions: reflection mode, liquid nitrogen-cooled mercury-cadmium telluride detector, 4000-560 cm^{-1} range, 4 cm^{-1} resolution, and 50 scans coaddition. All spectra were subjected to Kramers-Kronig transformation to transform them to absorbance spectra.[6]
- Specimens of the retrieved groups were carbon coated in a sputter coating unit (Bal-Tec, Vaduz, Liechenstein) and examined under a scanning electron microscope (Quanta 200, FEI, Hillsboro, Ore) equipped with an energy-dispersive X-ray microanalysis system, a silicon (lithium) energy dispersive spectrometry detector (Sapphire CDU, EDAX, Mahwah, NJ), and a super ultra thin berylium window. Elemental microanalysis was performed under 25 kV accelerating voltage, 100 µA beam current, 500 X original magnification with a 0.26 X 0.26 mm sampling window, 100-second acquisition time, and 30%-40% dead time. The quantitative analysis of the %wt concentration of the probed elements was performed by nonstandard analysis and atomic number, absorption, fluorescence correction routines by using the Genesis 3.5 software (EDAX, Mahwah).
- The Vickers hardness (HV_{200}) of the reference and retrieved specimens was assessed by using a microhardness tester (HMV-2000, Shimadzu, Tokyo, Japan) with a 30-g load and 15-second testing period. Four measurements were performed on 3 randomly chosen buccal areas of 3 appliances of each group. For technical reasons, related testing was limited to plane areas of the buccal segment.

Assessment of leaching

Two sets of maxillary and mandibular appliances—reference and intraorally xposed—were placed in 75% (v/v) ethanol-25%water immersion medium for 2 weeks at 23°C to simulate accelerated aging as proposed previously.[5] The substances leached in the immersion solution were then characterized with gas chromatography-mass spectrometry (GC-MS) with a GC-MS unit (Saturn 2000, Varian, Palo Alto,

Calif) equipped with an electron impact ionization detector. Approximately 10 mL of the samples was extracted with 10 mL HPLC-grade dichloromethane, and the extract was dried over anhydrous sodium sulfate. A PB-5 column of 30 m in length was used with the carrier gas at a flow rate of 30 cm/msec. The column program was 5 minutes at 40°C, 140°C intermediate temperatures, at a rate of 5°C per minute, 290°C final temperature at 10°C per minute, and 20 minutes holding time.

Results

Figure 1 depicts an optical microscopic image of a reference Invisalign specimen. After intraoral exposure, significant surface morphological changes were noted, including distortion and cracking (Fig 2, *A* and *B*), formation of amorphous integuments that masked the characteristic appliance structure (Fig 2, *C*), and regions of calcified integuments (Fig 2, *D*).

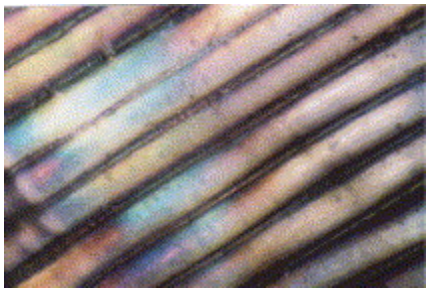

Fig 1. Polarized reflected light image of reference Invisalign appliance. Original magnification 20×.

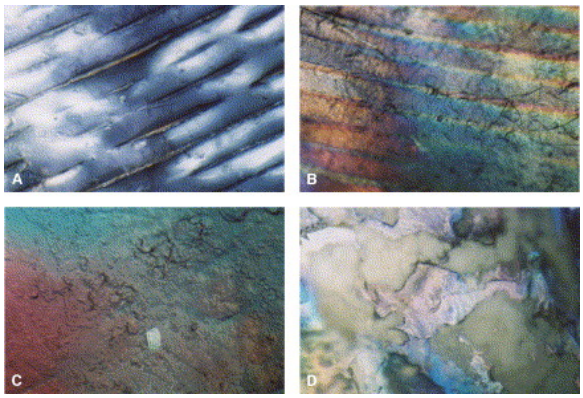

Fig 2. Polarized reflected light image of Invisalign appliances after intraoral exposure. **A,** Minimal distortion of surface serrations, **B**,surface cracking, **C**, absorption of an amorphous organized integument, and **D**, calcified regions. Original magnification, 20 X.

FTIR microscopic analysis of reference specimens confirmed the structure of 1,6 hexanediol methylene diphenyl diisocyanate. Spectra of retrieved specimens showed an irreversibly adsorbed proteinaceous film mainly composed of alcohol, amine, and amide groups (data not shown). In the same specimens, evidence of calcium phosphates was found, implying calcified integuments.

Scanning electron microscope and energy dispersive spectrometry X-ray microanalysis confirmed the presence of calcium and phosphorus in the calcified regions of the specimens, along with sodium, potassium, sulphur, and chlorine (data not shown).

The Vickers hardness of the buccal areas of in vivo aged and reference samples showed a substantial difference, indicating a ratio of retrieval-to-reference hardness of 5.2 (SD = 2.4).

The GC-MS spectra indicated no leaching from the materials. In addition, an identical spectrum pattern was obtained from the retrieved material.

Discussion

Invisalign appliances were found to be composed of polyurethane with
added methylene diphenyl diisocyanate and 1,6 hexanediol. The diphenyl structure provides stability and sufficient reactivity to form a polymer free of byproducts. This effect was confirmed by the GC-MS analysis of the solvent extracts; it showed no residual monomers or oxidative byproducts, even though the polar storage conditions in the ethanol/water solution were much more aggressive relative to the oral environment. Nevertheless, the immersion test might not reflect the degradation potential of these appliances in vivo.[7, 8, 9] This might be attributed to the fact that during intraoral service the material is subjected to potent abrasion from chewing action, along with attrition induced by the consumption of acidic beverages and the action of enzymes. At pronounced areas such as cusp tips, the movement of opposing teeth during mastication leads to abrasive wear, detachment of particles and gradual levelling of the appliance surfaces. This, however, does not result in cessation of the abrasion, because of the frequent renewal of the appliance sets, which induce another circle of abrasion. The fate of these particles and their biological properties are unknown. In general, particulate forms of polymers might have much higher biological action relative to bulk material because of their increased surface-to-volume ratio and the resultant increased reactivity with the surrounding environment. In the field of orthopedic biomaterials, where this issue has been exhaustively explored, it has been shown that the inert biological performance of ultra high molecular weight polyethylene acetabular sockets results in an inflammatory action induced by particle formation of worn materials.[11] Although the orthopedic application might be considered more invasive than the passive placement of aligners, the repeated use of new Invisalign appliances renders the characterization and quantitative aspects of the particle formation of critical importance.

Retrieval analysis is considered a first step in approaching the complex intraoral interaction pattern.[10] In this study, significant morphologic changes after in vivo exposure of aligners were identified, including cracking, wear of contact points, adsorption of proteinaceous material, and regional calcification of stagnation points such as central fissures. Calcification of proteinaceous biofilms formed on biomaterial surfaces exposed to body fluids has been considered a nonspecific mechanism of calcium precipitation.[12] X-ray microanalysis of selective regions demonstrated an almost uniform distribution of potassium, sodium, chlorine, and sulphur while others were mainly composed of calcium and phosphorus. The localized distribution of calcium and phosphorus in the same granular deposits is consistent with previous findings showing a delay in initiation of the biofilm calcification process.[13] Protein adsorption might induce entropically favorable conformational changes that, under localized conditions, can act as nuclei for forming small-atom microcrystalline deposits from sodium, potassium, and chlorine that are abundant in the oral environment. These deposits might dissolve under pH fluctuations, facilitating macromolecular displacement reactions, and thereby altering the pattern of the developing adsorption process.[14, 15] Once calcium precipitation begins, the size and the valence of the atoms might stabilize the structure by forming calcium phosphates under ordinary conditions.[16]

Changes in the Vickers hardness might be explained on the basis of alteration in the crystallinity of the appliance under cold work produced by masticatory loads. Whether this increase in hardness has any effect on the mechanotherapy by acting as a stiffening mechanism, interfering with the force delivery by the appliances, requires further study.

Conclusions

Retrieved Invisalign appliances demonstrate substantial morphological variation in relation to new specimens, involving abrasion at the cusp tips, adsorption of integuments at stagnation sites, and localized calcification of the biofilm developed during intraoral service.

An increase in hardness of the buccal segments of the retrieved appliances was found; this could mainly be attributed to cold work during mastication. The clinical implication of this effect in the force delivery of the appliance requires further study.

In vitro aged appliances were found not to release traceable monomers or byproducts after immersion in an ethanol-water solvent. The aging pattern of these appliances intraorally involves abrasive wear arising from mastication, and, thus, no definitive consensus on their reactivity and biological properties can yet be established.

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