

Structural conformation of *in vitro* and *in vivo* aged orthodontic elastomeric modules

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SUMMARY The objectives of this study were to investigate the structural characteristics of open and closed elastomeric modules in the as-received condition, and following (a) 24-hour *in vitro* loading determined as a 50 per cent extension relative to their original length; (b) 24-hour intra-oral exposure; (c) 3-week intra-oral exposure. Ten specimens of each type (open and closed) of the three brands of elastic chains were included in the study (total of 60). All specimens were subjected to (a) optical transmission microscopy utilizing phase contrast and polarized light modes, (b) micro MIR FT-IR spectroscopy, and (c) scanning electron microscopy and electron probe X-ray microanalysis.

Stretched elastomers demonstrated a honeycomb pattern of filament detachment corresponding to strained areas. The chains possessing a well-differentiated inter-modular link presented higher strain concentration gradients located in the link. Phase contrast imaging revealed that the stress adsorption mechanism of these materials involved macromolecular chain orientation and elongation co-axially to the applied load. The specimens retrieved after 24 hours had acquired a proteinaceous film rich in alcohol groups with minimal evidence of phosphate mineralization, while the 3-week retrieved specimens presented precipitation of Ca- and P-forming calcium phosphates. The high protein content of the biofilm organized on the surface of these materials, as well as the calcification pattern found, were similar to a non-specific mechanism of film adsorption of biomaterials exposed to body fluids.

The results have clinical implications for aspects of retraction control through sliding mechanics with the use of elastomeric ligatures, and the potential detrimental effects on dental and periodontal tissues such as decalcification and gingival inflammation, respectively. In addition, the experimental protocols adopted for the *in vitro* study of the force decay of elastomerics should be modified to include parameters pertinent to adsorption phenomena and calcification processes documented to occur *in vivo*.

Introduction

Considerable advances have been recorded during the last two decades in the field of orthodontic materials technology associated with archwire, bracket manufacturing, and novel bonding techniques. This progress has contributed to the establishment of an orthodontic apparatus, which will reliably facilitate the application of forces of a predetermined magnitude, specific direction, and desirable duration in a predictable manner, with minimum substrate alterations induced. Nonetheless, the armamentarium of elastic modules has remained, at large, the same.

Elastomerics, in general, are polyurethanes, thermosetting polymer products of a step-reaction polymerization process, possessing a $-(NH)-(C=O)-O-$ unit (Billmeyer, 1984). Despite the fact that most of the orthodontic elastomeric modules currently available share similar fabrication methods, significant variations have been presented with regard to their force decay characteristics and force relaxation patterns (De Genova *et al.*, 1985; Kuster *et al.*, 1986). These differences may be attributed to (a) processing variations in module manufacturing techniques involving cutting or injection-moulding of the

raw material; (b) effects induced from various additives incorporated in the final product; (c) different morphological (ellipsoid or circular modules) or dimensional characteristics (presence or absence of inter-modular link) of the chains. Similarly, such a discrepancy has been documented in the literature through several protocols adopted for the evaluation of the force degradation rates of elastomerics, involving dry or wet testing states (Rock *et al.*, 1986), including water, artificial saliva, or fluoride media (von Fraunhofer *et al.*, 1992), with varying temperatures (Brooks and Hershey, 1976), decreasing or steady force application (Lu *et al.*, 1993), and acidic or neutral pH (Ferriter *et al.*, 1990).

Nevertheless, the main factor that distinguishes the oral environment is the presence of complex oral flora and its by-products, as well as the accumulation of plaque on the material. This cannot be simulated under current *in vitro* research methodological approaches. The significance of this particular parameter has been validated in the literature, and found capable of inducing substantial alterations in the structure and surface properties of an array of materials exposed in the oral cavity extending from restorative composite materials (Freund and Munksgaard, 1990), to orthodontic adhesives (Matasa, 1995), and archwires (Oshida *et al.*, 1992). In the light of evidence suggesting that polyurethanes are not inert materials, and are highly affected by heat, moisture, and prolonged contact with enzymes, this hypothesis gains special interest. Water has been found to act as a plastisizer of the polymeric material by weakening of intermolecular forces, leading to chemical degradation (Huget *et al.*, 1990). Analysis of leached substances from stretched elastomeric modules intended for orthodontic applications has shown increased leaching following 7 days of loading in specimens immersed in water. This effect is evidently due to the presence of ester or ether backbone linkages, which are highly susceptible to hydrolysis and are the first compounds to be affected by water attack (Schollenberger and Stewart, 1971). Thus, intruding water molecules may weaken the material, causing a reduction in the load required to maintain a fixed extension. This event is expressed as force decay in force

relaxation experimental set-ups (Phua *et al.*, 1987). The hypothesis tested in this study was that the complex conditions present in the oral cavity and the increased plaque accumulation substantially alter the surface properties and structural conformation of the elastomerics.

The objectives of this investigation were to:

- (1) study the microstructural alterations;
- (2) identify possible compositional variations;
- (3) investigate the strain distribution of elastomeric chains in the as-received condition and following initiation of a standardized strain (50 per cent of original length) for 24 hours *in vitro*.

In addition, the aforementioned variables together with information on the composition of adsorbed species were examined after intra-oral functional exposure for periods of 24 hours and 3 weeks, respectively.

Materials and methods

Two types (open and closed chains, i.e. with and without inter-modular link) of three brands (Generation II, Ormco, Glendora, CA, USA; Dentaureum elastic chain, Dentaureum, Pforzheim, Germany; and Alastik 3M/Unitek, Monrovia, CA, USA) of elastomeric modules were included in the study.

Specimens were prepared by cutting a multiple series of each type and brand containing 10 samples each (total 60), having an equal number of loops (6) from the spools, with the use of a sharp ligature cutter. The choice of the number of loops as opposed to standardized length of chain was based on the variability of shape and design noted among the brands selected. This precluded the parameter of initial length as a reliable normalization factor for the fabrication of specimens. Care was taken to avoid extended handling during cutting as this might have incorporated stresses in the material prior to testing. These specimens were measured and divided into four groups.

The first group served as representative of the as-received state. The second group was subjected to a 24-hour static fatigue test by maintaining steady strain in air, determined as

50 per cent of its original length. The third was used for the intra-oral application of modules elongated at approximately 50 per cent extension relative to the as-received state. Specimens comprising the latter group were retrieved following 24 hours of exposure to the oral cavity of one patient with good oral health under orthodontic treatment with edgewise, 0.018-inch, Hilgers prescription, Ormco diamond brackets, and receiving no medication. This facilitated comparison between material alterations induced by *in vitro* stretching, and intra-oral extension during the same time period. Lastly, a fourth group consisted of specimens retrieved after a 3-week period of exposure to the oral environment of patients receiving orthodontic therapy by the same treatment provider with brackets identical to the 24-hour retrieval experiment. The modules were elongated approximately 50 per cent relative to the as-received state. These specimens after retrieval were rinsed with copious amounts of distilled water to remove the loosely bound intra-orally formed integuments.

Representative samples from the four groups were subjected to multi-technique characterization employing:

1. Brightfield optical transmission microscopy utilizing a microscope (Microphot Nikon, Kogaku, Tokyo, Japan) operating in phase contrast and polarized light modes for the investigation of morphological variations and residual stress gradients within the modules.
2. Micro-multiple internal reflectance Fourier transform infra-red (micro-MIR FTIR) spectroscopy for the characterization of the *in vivo* changes in molecular composition induced in elastomeric surfaces. Spectra acquisitions were performed on an FTIR spectrometer (PE 1760 X, Perkin Elmer Corp. Norwalk, CT, USA) operating under the following conditions: 4000–400/cm range, 4/cm resolution, 50 scans co-addition, KRS-5 mini-crystal of 45 degrees edge and 14 internal reflections, 2.5 μm depth of analysis at 1000/cm.
3. Scanning electron microscopy and wave-length dispersive electron probe microanalysis (SEM–WDS) to assess the elemental composition

of the morphological alterations observed after intra-oral exposure. For this purpose, specimens were vacuum-coated with a thin layer of graphite and examined under an electron probe micro-analyser (JXA 733 Superprobe, JEOL Ltd., Tokyo, Japan). Secondary electron images (SEI) and back-scattered electron images for topography (BEI-TOPO) and composition (BEI-COMPO) were recorded at 20 kV and 8 nA. Elemental analysis was performed with two spectrometers equipped with PET, TAP, LIF, and STE crystals utilizing area scan mode analysis.

Results

Figures 1 and 2 depict the microscopic appearance of as-received (group a) and *in vitro*-stretched (group b) elastomeric modules under polarized light. Stretching induced the formation of isochromatic (colour) and isoclinic (dark)

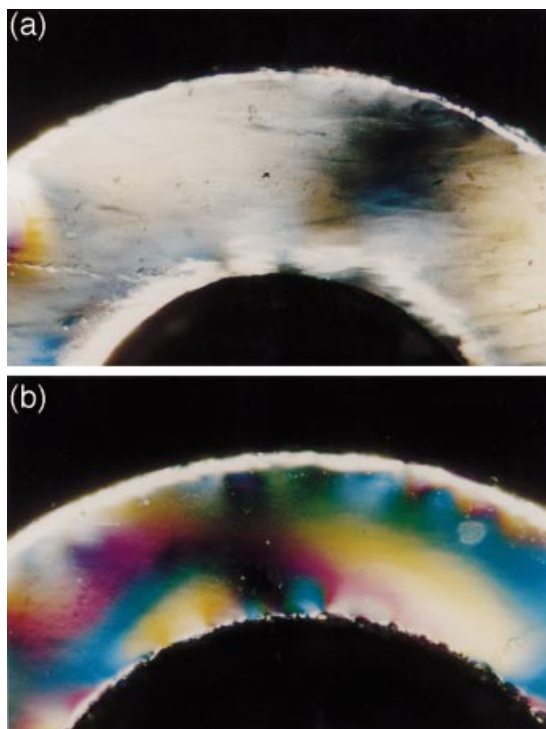


Figure 1 Transmitted polarized light images of ring-shaped modular units of a Generation II (Ormco) elastomeric module as received (a) and following *in vitro* stretching (b). (Brightfield, crossed polarizers $\times 20$ original magnification.)

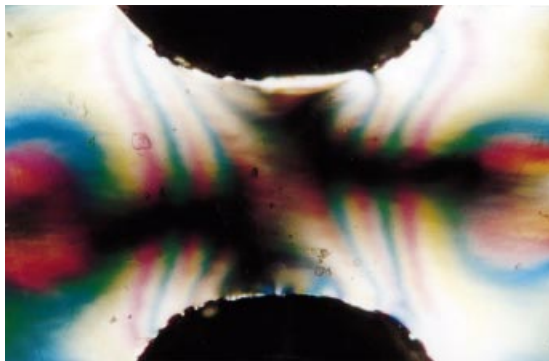


Figure 2 Transmitted polarized light images of an inter-modular link of an Alastik (3M/Unitek) elastomeric module after *in vitro* stretching. Note excessive residual stresses compared with the ring-shaped modular unit of Figure 1b. (Brightfield, crossed polarizers, $\times 20$ original magnification.)

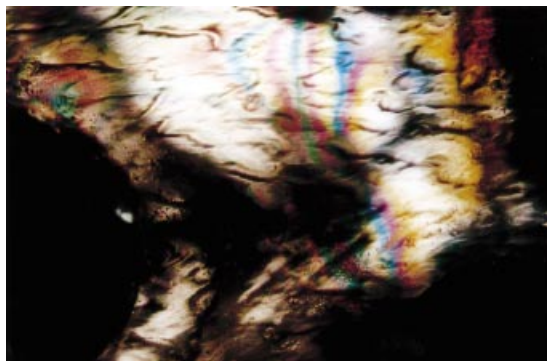


Figure 3 Transmitted polarized light image of a retrieved Dentaurem inter-modular link after 3 weeks intra-oral placement. Note similarity in the isochromatic fringe pattern and lack of resolution due to alterations in transmittance characteristics. (Brightfield, crossed polarizers, $\times 20$ original magnification.)

fringes related to the stress intensity and stress direction vectors, respectively. The large number and closer arrangement of isochromatic fringes located at inter-modular links (Figure 2) implies that this region exhibited high residual stress intensity and concentration (Figure 1b). Although the direction of the *in vitro* tensile force applied was co-axial to the longitudinal module axis, the residual stresses developed at links and rings were directed vertically to the material margins as noted from the symmetric direction of the isoclinic fringes.

Retrieved specimens demonstrated similar residual stress distribution patterns (Figure 3). However, the interaction of the elastomers with the factors emanating from the oral environment strongly modified the optical transmission characteristics of the modules, diminishing polarized light resolution. Phase contrast microscopic examination revealed important changes in module structure (Figure 4a–d). The original elastomer modular structure, comprising mainly of partially orientated fissures and ridges (Figure 4a), was transformed to a smooth structure. This was highly orientated to the direction of the applied force following stretching or intra-oral exposure. Retrieved specimens showed smoothing of the elastomer structure with marginal cracks developed towards bulk material (Figure 4b) and irreversible adsorption of integuments formed

intra-orally (Figure 4c). Evidence of permanent deformation was found in chain rings of retrieved materials associated with the engagement of the material underneath the bracket wings (Figure 4d) for the entire sample size. All specimens were found to share the pattern identified.

Representative spectra from the micro MIR-FTIR study are shown in Figures 5 and 6. After 24 hours of intra-oral placement, the elastomer surfaces were modified from irreversible adsorption of proteinaceous matter characterized from $-\text{OH}$ (3600–3300/cm), $-\text{NH}$ (3300–3200/cm), CH_3-CH_2- (2900/cm and 1400/cm), amide I (1684/cm), amide II (1540/cm), amide III (1250/cm), $-\text{CH}-\text{OH}$ (1180/cm) and $-\text{CH}_2-\text{OH}$ (1095/cm) groups with slight evidence of mineralization as shown from the $-\text{PO}_4$ group peak at 1065/cm (Figure 5, subtracted spectrum). Following 3 weeks *in vivo* the spectra of elastomeric surfaces were found to be predominated by peaks assigned to proteinaceous matter exhibiting excessive mineralization as indicated by the increased intensity of the orthophosphate peak at 1060/cm (Figure 6). Acid phosphate (545/cm) and carbonate groups (870/cm) were also identified. Original elastomer peaks were almost completely masked by the acquired biofilms. This effect was common for specimens of all types and brands tested.

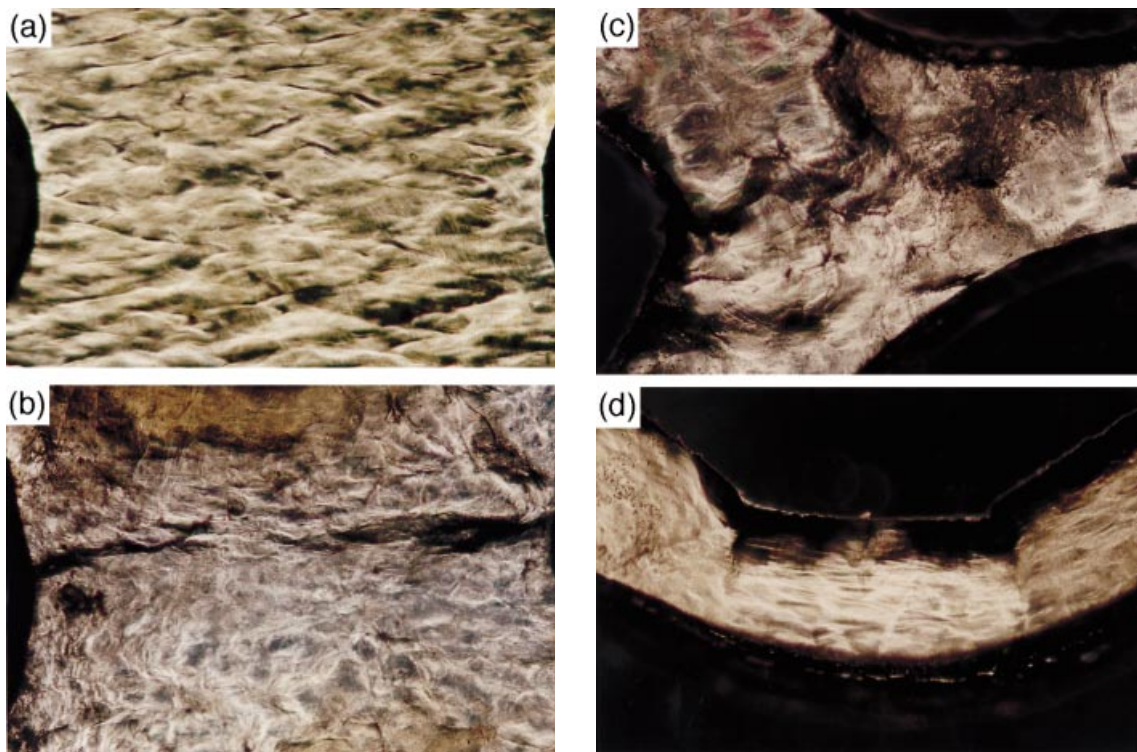


Figure 4 Phase contrast images of Generation II (Ormco) elastomeric modules. (a) As received. (b) After 24 hours intra-oral exposure. Note changes in texture and crack propagation. (c) After 3 weeks intra-oral exposure. Note deposit build-up. (d) Permanent deformation of a ring-shaped modular unit as a result of its engagement in the bracket wings. (Brightfield, phase 2, $\times 20$ original magnification.)

Figure 7 (a–d) shows the results of the electron microprobe analysis of a representative elastomeric module retrieved after 24 hours of intra-oral exposure. The SEI (Figure 7a) revealed the development of flat coherent interconnected deposits orientated by deep fissures, which extended to the elastomer surface. Onto these deposits some granular structures and pores were identified. The BE-COMPO image (Figure 7b) illustrated a rather uniform grey level of the deposits with well defined white regions corresponding to the granular structure of the SEI. The white margins observed in some deposits arose from the edge-effect produced from electron scattering due to height differences between elastomer and deposit surface levels. Figure 7c presents the elemental X-ray mapping of K corresponding to the BE-COMPO image of Figure 7b. It is interesting to note that the distribution of K follows in detail the deposit outline.

Similar distributions were recorded for Na, Cl, and S at a reduced intensity scale, while the distribution of Ca (Figure 7d) was related to the granular structures found on the flat deposits. The same pattern was obtained from the P distribution, which presented lower signal intensity. Identical trends were found for the three brands included in the study.

After 3 weeks intra-oral exposure, the deposits were more organized, the height differences between deposits and elastomer surfaces were increased, and the morphological appearance of the deposits was rough, characterized by the abundance of aggregated particles (Figures 8 a–c). The distribution patterns of K, Na, and Cl followed that of the deposits, but at a considerably lower intensity than in the 24-hour intra-oral exposure group (Figure 8d). No S was detected, while the distribution of Ca and P corresponded exactly to the elastomer area covered by the

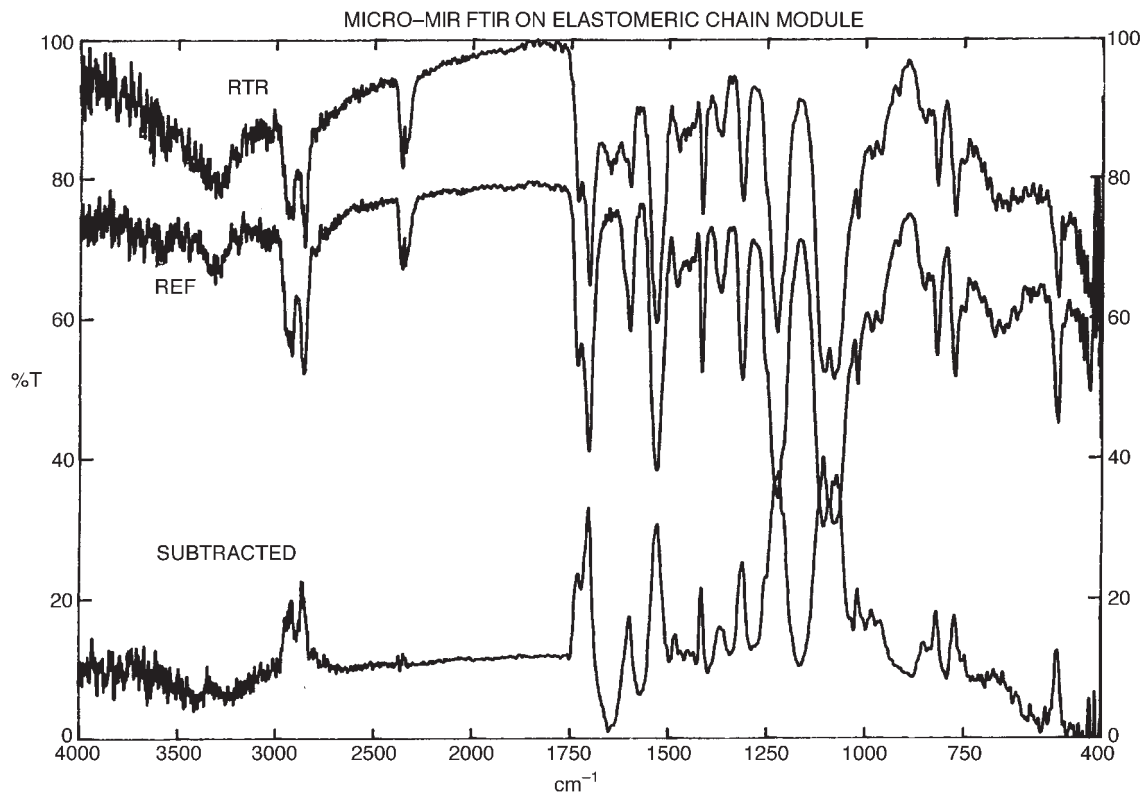


Figure 5 Micro MIR-FTIR spectra of an Alastik (3M/Unitek) chain module as received (control group-REF), following 24 hours intra-oral exposure (retrieved-RTR) and the resulting subtraction spectrum.

deposit (Figure 8e). The concentration of Ca was greater than that of P as the Ca signal intensity recorded was much higher.

Discussion

The characteristic pattern of fringe formation in the polarized light microscopic examination occurred as a result of alterations of the polarized light by the residual internal stresses into two waves that travel at different velocities (Durelli and Riley, 1965). This allows for the estimation of the strain intensity and direction developed in the material. In the elastic chains possessing well-differentiated inter-modular links (open chains), the direction and intensity of the strain produced corresponded better to the link extension pattern. This was in contrast to the

closed elastomers, where the strain developed in the modular rings was much higher. Moreover, as the latter was subjected to additional stresses arising from the engagement of the module into the brackets, a higher tendency to force degradation or ring failure was anticipated in the closed elastomer modules.

Phase contrast imaging confirmed that the stress adsorption mechanism in these materials seems to be that of macromolecular chain orientation and elongation, co-axially to the applied load. However, regional discontinuities emanating from the design of chains may locally exaggerate stress effects, leading to a micro-tearing pattern and establishment of microfractures, which propagate from margins to bulk material. This failure pattern involves fracture lines directed perpendicular to the elastomeric margins,

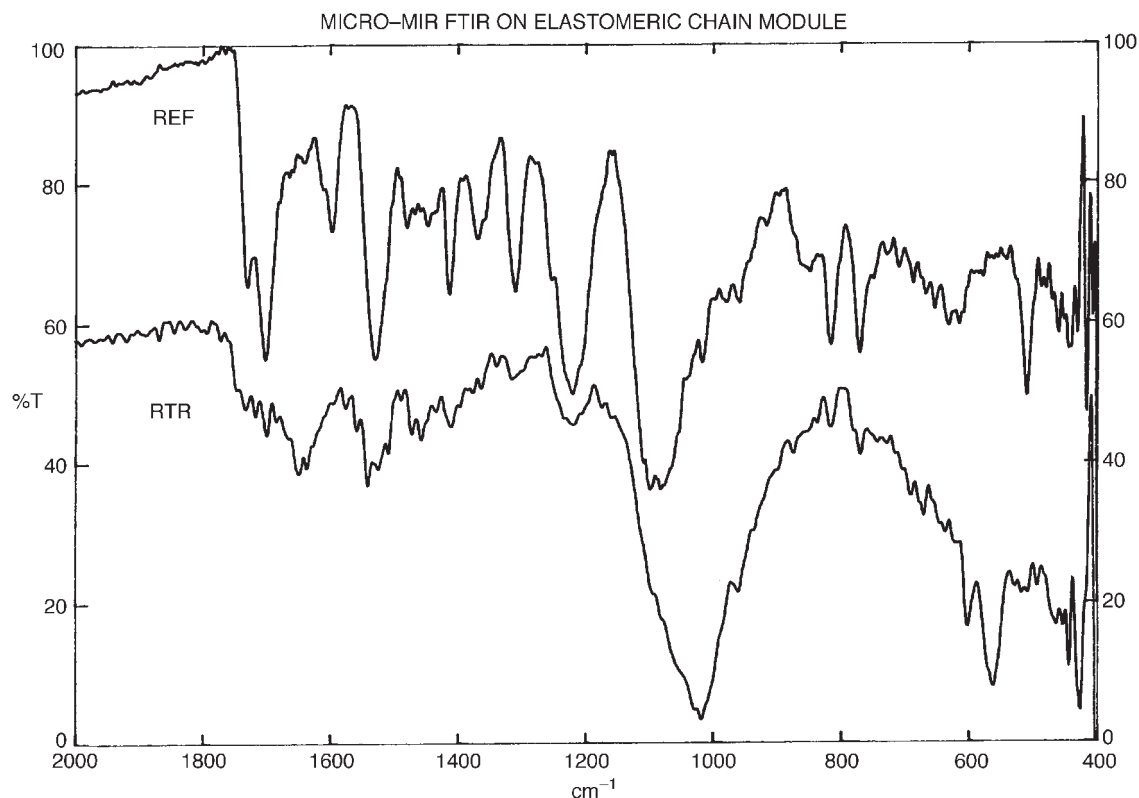


Figure 6 Micro-MIR FTIR spectra of an Alastik (3M/Unitek) chain module before (control group-REF) and after 3 weeks intra-oral placement (retrieved-RTR).

following the residual stress vectors, as was demonstrated from the direction of the isoclinic fringes in the polarized light images.

The micro-MIR FTIR spectroscopic analysis of untreated elastomeric modules revealed variations in their molecular composition implying the use of different poly-(ester) urethane raw materials in the fabrication processes. These variations were mainly assigned to differences in the peak intensity between free urethane and ester C=O stretching vibrations (1730/cm and 1284–1222/cm) and hydrogen bonded ester C=O vibrations (1704/cm and 1222/cm). This is consistent with the use of two raw materials with different molecular unit structures (Barbucci and Magnani, 1992). Minor changes were found in the intensity of some groups in spectra obtained from *in vitro* stretched specimens relative to the

untreated controls, which may be attributed to macromolecular orientation effects due to plastic deformation of the chain. However, the most prolonged changes in molecular composition occurred after intra-oral exposure of these materials. After 24 hours exposure, the elastomeric surfaces were covered by a non-continuous proteinaceous film rich in alcohol groups with minimal evidence of phosphate mineralization. The increased intensity of the peaks at 3600–3300/cm and 3300–3200/cm regions show that this film was organized mainly by hydrogen bonded –OH and –NH groups of proteins and carbohydrates, a finding which is in agreement with previous reports (Baier and Glantz, 1978). The role of hydrogen bonding is very important in polyurethanes since these materials may act both as H-donors, through –HN groups, or as

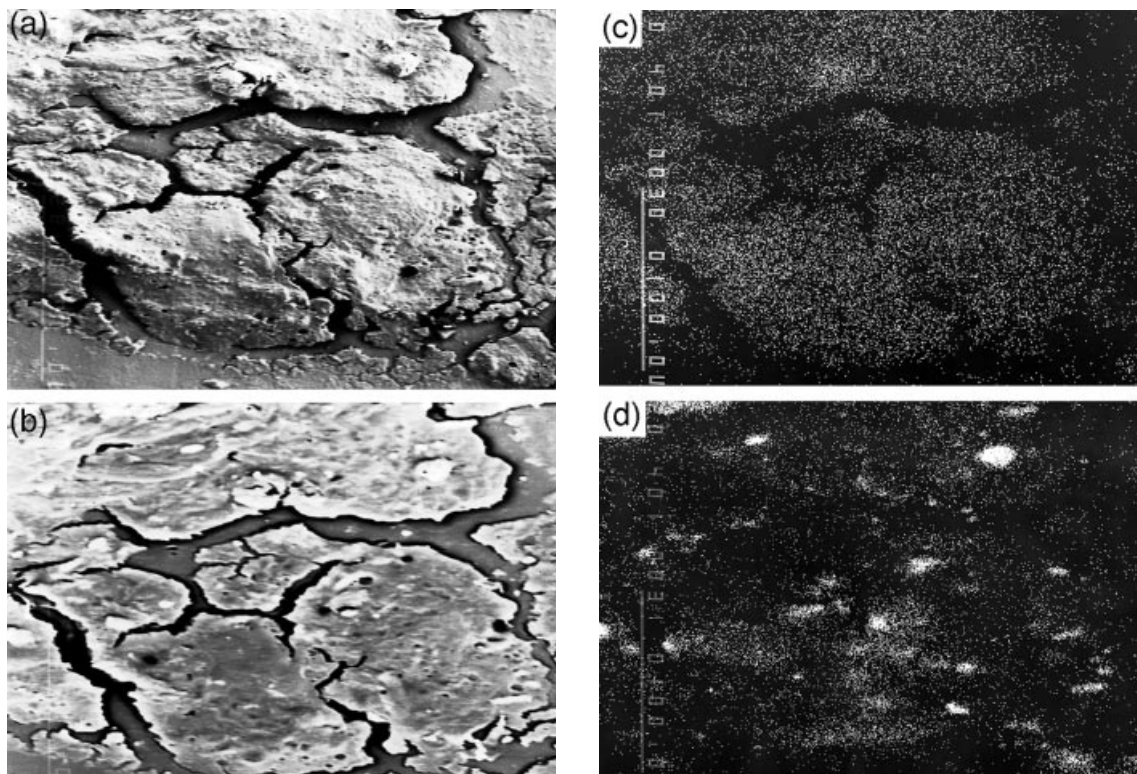


Figure 7 SEM-WDS X-ray microanalysis of a Generation II (Ormco) chain module retrieved after 24 hours intra-oral placement. (Original magnification $\times 400$, bar 100 μm .) (a) SEI. (b) BEI-COMPO. (c) X-ray image of K. (d) X-ray image of Ca.

H-acceptors, through C=O groups (Merrill, 1987). Following 3 weeks intra-oral exposure, elastomeric surfaces were found to be covered by well-mineralized proteinaceous films composed of calcium phosphates with carbonate and acid-phosphate impurities. This pattern of precipitation organization was consistent in the materials examined, and seemed to dominate regardless of specific chain brand or type.

Calcification of proteinaceous biofilms formed on biomaterial surfaces exposed to body fluids has been considered a non-specific mechanism of calcium precipitation (Vasin *et al.*, 1998). More details on this process are provided by the combined SEM-WDS micro-analytical study. Compositional back-scattered electron images presented important information on the extent of mineralization of selective features based on the grey levels observed and on the correlation of regional differences in atomic number with the elemental composition patterns recorded.

X-ray microanalysis of regions demonstrating low contrast and a wide range of grey level, showed an almost uniform distribution of K, Na, Cl and S, while high contrast regions imaged as soft-white areas, were mainly composed of Ca and P. The localized distribution of Ca and P within the same granular deposits formed onto low atomic number crystallites which was observed after 24 hours intra-oral exposure, is consistent with previous findings showing a delay in initiation of biofilm calcification process (Glantz, 1980). It seems that protein adsorption on elastomeric surfaces induces entropically favourable conformational changes which, under localized conditions, may act as nuclei for the formation of small-atom microcrystalline deposits formed from Na, K, Cl, which are present in abundance in the oral environment. These deposits may dissolve under pH fluctuations facilitating macromolecular displacement reactions, thereby altering the pattern of the developing adsorption

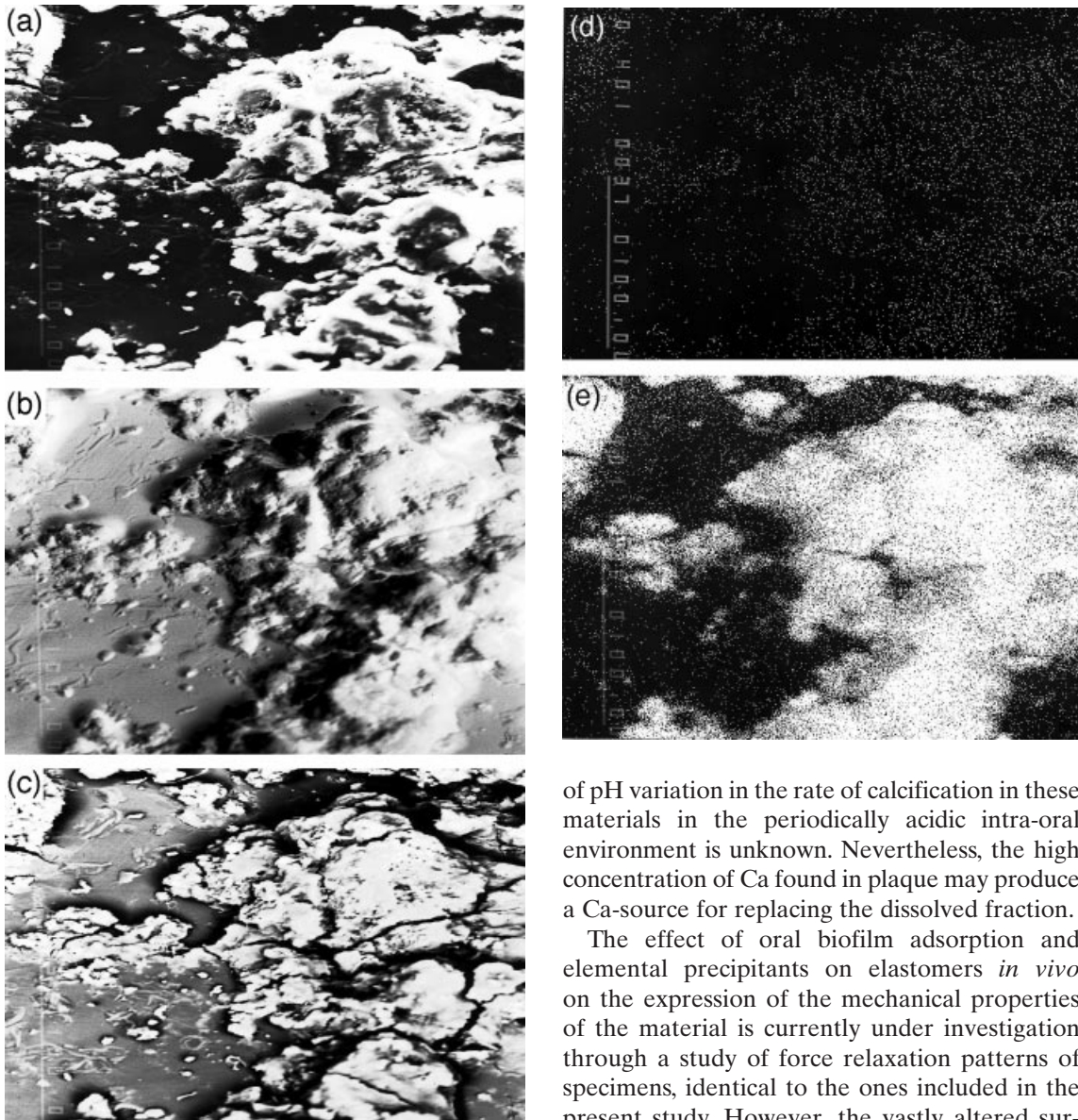


Figure 8 SEM-WDS X-ray microanalysis of a Generation II (Ormco) chain module retrieved after 3 weeks intra-oral placement. (Original magnification $\times 400$, bar 100 μm .) (a) SEI. (b) BEI-TOPO. (c) BEI-COMPO. (d) X-ray image of K. (e) X-ray image of Ca.

process (Leininger *et al.*, 1987). Once Ca precipitation is initiated, the size and valence of the atoms stabilizes the structure by formation of calcium phosphates under ordinary conditions (Kasemo and Lausmaa, 1991). The implication

of pH variation in the rate of calcification in these materials in the periodically acidic intra-oral environment is unknown. Nevertheless, the high concentration of Ca found in plaque may produce a Ca-source for replacing the dissolved fraction.

The effect of oral biofilm adsorption and elemental precipitants on elastomers *in vivo* on the expression of the mechanical properties of the material is currently under investigation through a study of force relaxation patterns of specimens, identical to the ones included in the present study. However, the vastly altered surface structure and composition of the retrieved modules is strongly indicative of the severity of the changes induced. This leaves no doubt about the compromised performance of the materials. Additional research is being conducted with the object of differentiating the interaction pattern, i.e. synergism, of the effects of exposure to an aqueous environment and strain, since evidence in the literature suggests that initial alteration of the material's structure may occur through hydrolytic degradation (Huget *et al.*, 1990).

The clinical implication of the information reported in this article pertains to aspects of retraction mechanics. Sliding mechanics with the use of elastic chains on NiTi super-elastic wires, which presumably allows for convenient screening with intervals between appointments exceeding 2 months, may require substantially shorter screening time periods to facilitate reactivation of the force applied. In addition, the plaque accumulated on the exposed material may have detrimental effects on the surrounding hard and soft tissues, since its proximity to bracket margins may enhance the possibility of undesirable effects such as enamel decalcification or gingival inflammation.

Conclusions

The combined imaging and spectroscopic techniques disclosed the macromolecular chain orientation of all elastomeric modules under load and the progressive formation of a proteinaceous biofilm that underwent subsequent biomineralization by calcium phosphates.

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