Cytotoxicity, genotoxicity, and metal release in patients with fixed orthodontic appliances: A longitudinal in-vivo study

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Introduction: Treatment with fixed orthodontic appliances in the corrosive environment of the oral cavity warrants in-vivo investigations of biocompatibility. **Methods:** Eighteen control and 28 treated subjects were evaluated longitudinally. Four combinations of brackets and archwires were tested. Buccal mucosa cell samples were collected before treatment, and 3 and 6 months after appliance placement. The cells were processed for cytotoxicity, genotoxicity, and nickel and chromium contents. **Results:** In the treatment group, buccal mucosa cell viability values were 8.1% at pretreatment, and 6.4% and 4.5% at 3 and 6 months, respectively. The composite score, a calculated DNA damage value, decreased from 125.6 to 98.8 at 6 months. Nickel cellular content increased from 0.52 to 0.68 and 0.78 ng per milliliter, and chromium increased from 0.31 to 0.41 and 0.78 ng per milliliter at 3 and 6 months, respectively. Compared with the control group, the treated subjects showed significant differences for DNA damage and chromium content at 3 months only. **Conclusions:** Fixed orthodontic appliances decreased cellular viability, induced DNA damage, and increased the nickel and chromium contents of the buccal mucosa cells. Compared to the control group, these changes were not evident at 6 months, possibly indicating tolerance for or repair of the cells and the DNA. (Am J Orthod Dentofacial Orthop 2011;140:298-308)

The use of various combinations of metal alloys for prolonged durations in orthodontic patients warrants special consideration regarding their biocompatibility. The oral cavity is a complete corrosion cell, with many factors that enhance the biodegradation of orthodontic appliances.[1] Saliva acts as an electrolyte for electron and ion conduction, and the fluctuation of pH and temperature, the enzymatic and microbial activity, and the various chemicals introduced into the oral cavity through food and drink are all corrosion conductors.[2] The inherent heterogeneity of each metal alloy and its use with other alloys, the microsurface discontinuity, the forces acting on the appliances, and the friction between wires and brackets also add to the corrosion process. The literature includes many in-vivo[3-8] and in-vitro[9-16] studies documenting the corrosion of orthodontic appliances, and the release of metal ions is indisputable. It has been reported that metal ions are taken up by the adjacent oral tissues.[17-19]

As pointed out by Wataha,[20] the corrosion of an alloy is of fundamental importance to its biocompatibility because the release of elements from the alloy is nearly always necessary for adverse biologic effects such as toxicity, allergy, mutagenicity, and carcinogenicity. Alloy corrosion provides free ions that affect the tissues around it. There is little evidence that elements released from casting alloys contribute significantly to systemic toxicity. The cause of this might be explained by the low release of ions over time.

Metal tolerance and the amounts causing toxicity are not well understood. Metals are not biodegradable, and their sustained release might produce irreversible toxic effects from their accumulation in the tissues. Also, the increased exposure could limit the recovery time needed

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for cellular repair. Metal toxicity is governed by multiple factors, making it difficult to truly assess the levels that produce cellular damage.[21,22]

The general belief that there is no frank concern regarding the corrosion by-products released in orthodontic patients is not actually supported.[23,24] The literature on cancer research and metal toxicology includes many reports of the dangers of various metal ions.[21,25-27] Nickel and chromium ions, which are abundant components of most orthodontic alloys, are classified as chemical carcinogens.[28] The recent insight into the cellular and molecular mechanisms of metal toxicity might cause some concern when dealing with orthodontic appliances.[25,27,29] That is due to their direct prolonged contact with the oral tissues and their corrosion, resulting in the release of various types and amounts of metal ions.

Because most research on the amounts of metal ions released from orthodontic alloys has shown that they fall below the recommended daily dietary intakes of nickel and chromium,[3,9-13,15,16] this might be a false assurance of safety, since chronic low levels of metal ions can alter cellular metabolism and morphology, and produce inflammation and even DNA instability.[30-35] In addition, some in-vivo studies reported biologic toxicity in orthodontic patients.[18,36-38]

To test the biocompatibility (cytotoxic and genotoxic effects on human tissues) of fixed orthodontic appliances, a longitudinal controlled clinical trial was set up with 3 distinct hypotheses: (1) fixed orthodontic appliances do not have a toxic effect, (2) fixed orthodontic appliances do not have an effect on the cellular metal content in buccal mucosa cell samples, and (3) there is no difference between the effects of the various materials of fixed orthodontic appliances.

MATERIAL AND METHODS

Sixty subjects were included in this study. Forty patients required fixed orthodontic treatment, and 20 subjects served as the control group. The aims of the study and the method of cell collection were explained to all subjects, and written consent to participate was obtained. Treatment was started after the institutional ethical committee approved the protocol.

Two subjects from the control group and 8 patients dropped out of the study. The causes for not completing the 6-month study period were variable. Some subjects received medications, others underwent oral or general surgeries, others terminated treatment because of discomfort from the appliances, and 4 were excluded for loose brackets. Eighteen control (8 men, 10 women) and 28 treated subjects (6 men, 22 women) completed the study. The average ages were 21 years 6 months (± 3.3 years) in the control group and 20 years 2 months (± 4.4 years) in the treatment group.

The eligibility criteria for subject selection included nonsmokers; no oral diseases, systemic diseases, oral restorations or prosthetics; clinically healthy oral mucosa; no previous orthodontic treatment; no occupational exposure to metals; not receiving any medications or supplements; no radiographic examination during the previous 6 months; and no known allergy to costume jewelry, watches, or sources of nickel and chromium. Subjects were initially screened with a questionnaire to check whether they fit the criteria of the study. They were then clinically assessed for normal oral mucosa.[39] The orthodontic patients were all treated with fixed orthodontic appliances in both arches. The appliances consisted of 4 bands (3M Unitek, Monrovia, Calif) on the first permanent molars, brackets, and 1 type of archwire material throughout the study.[20] The archwires were replaced at 6-week intervals. A total of 4 wires were used for each patient over the 6-month period. The sizes of the archwires were 0.012, 0.014, 0.016, and 0.018 in. The archwires were fixed with 0.01-in stainless steel ligature wire (Leone, Florence, Italy). The compositions of the material alloys are given in Table I.

The patients were divided into 4 groups according to the combination of brackets and archwires. The brackets used were standard stainless steel (American Orthodontics, Sheboygan, Wis) and equilibrium titanium (Dentaurum, Ispringen, Germany), and the archwire materials were stainless steel and nickel-titanium alloys (both, GAC International, Bohemia, NY). Group 1 had stainless steel brackets and stainless steel wires (StSt-StSt). Group 2 had stainless steel brackets and nickel-titanium wires (StSt-NiTi). Group 3 had titanium brackets and stainless steel wires (Ti-StSt). Group 4 had titanium brackets and nickel-titanium wires (Ti-NiTi).

Before the start of the study, all subjects were instructed to continue brushing but not to use toothpastes and mouthwashes containing fluoride or chlorhexidine because these have been reported to increase DNA damage in buccal mucosa cells.[40,41] A sampling schedule was set up that allowed all subjects to be measured over the same period of time, not consecutively, to prevent the effect of seasonal changes on the assessment of DNA.[42]

Buccal mucosa cells were evaluated before treatment (T0), and at 3 months (T1) and 6 months (T2) after appliance placement. The cells were harvested, according to the method of Nia et al[43] by gentle scraping of the internal part of the right and left cheeks with a wooden tongue depressor. Gentle scraping was required to prevent a heterogeneous cell sample.[44] Each tongue depressor was stirred in a 2-mL tube (Eppendorf,