



# In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells

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Interest in the amount of metal ion intake from dental alloys has grown. Fixed orthodontic appliances usually include brackets, bands, and archwires made of stainless steel, nickel-titanium, or nickel-cobalt alloys, and these can release metal ions. The purpose of this study was to investigate the biocompatibility in vivo of fixed orthodontic appliances, evaluating the presence of metal ions in oral mucosa cells, their cytotoxicity, and their possible genotoxic effects. Mucosa samples were collected by gentle brushing of the internal part of the right and left cheeks of 55 orthodontic patients and 30 control subjects who were not receiving orthodontic treatment. The cells were immediately prepared for cell viability and the comet assay. Nickel and cobalt cellular content was quantified by inductively coupled plasma mass spectrometry (ICP-MS). The results indicate that nickel and cobalt concentrations were 3.4-fold and 2.8-fold higher, respectively, in the patients than in the controls; cellular viability was significantly lower in the patients than in the controls, and there was a significant negative correlation with metal levels. The biologic effects, evaluated by alkaline comet assay, indicated that both metals induced DNA damage (more cells with comets and apoptotic cells). There were significant positive correlations between (1) cobalt levels and the number of comets and apoptotic cells, (2) nickel levels and number of comet cells, and (3) cobalt levels and comet tails. This study corroborates that nickel and cobalt released from fixed orthodontic appliances can induce DNA damage in oral mucosa cells. (Am J Orthod Dentofacial Orthop 2003;124:687-94)

Several studies have investigated whether orthodontic appliances release metal ions through emission of electro-galvanic currents, with saliva as the medium or through continuous erosion over time.<sup>1,2</sup> The oral environment is particularly ideal for the biodegradation of metals because of its thermal, microbiologic, and enzymatic properties. Intraoral fixed orthodontic appliances include brackets, bands, and archwires that are made of alloys containing nickel, cobalt, and chromium in different percentages. At present, the different types of orthodontic archwires

contain 15% to 54% nickel, 20% to 30% chromium, and 40% to 60% cobalt.<sup>3,4</sup> Therefore, data on the biocompatibility of alloys are of great interest.

Nickel is a strong immunologic sensitizer, although nickel sensitivity has been reported to be lower in subjects who have received orthodontic treatment; perhaps they develop immunological tolerance over the long period of treatment.<sup>5,6</sup> On the other hand, chromium and cobalt ions can also cause hypersensitivity, dermatitis, and asthma.<sup>7-9</sup> These metals can induce other adverse biologic effects, such as cytotoxicity, and they are suspected genotoxic agents.<sup>10-12</sup> Many metal compounds are carcinogenic to animals or humans; their mechanisms are not overall known, but 1 pathway might be the involvement of the metals with DNA interaction, either directly or indirectly. Several metals have been shown to have cogenotoxicity; 2 mechanisms seem to be predominant: the generation of oxidative DNA damage and the interference with DNA repair and DNA replication processes.<sup>13,14</sup>

Recently, an electrophoretic technique capable of detecting DNA single strand breaks and alkali labile sites in individual cells has been developed by Singh et al.<sup>15</sup> The importance of the single-cell gel electrophoresis or comet assay comes from its ability to evaluate

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Submitted, January 2003; revised and accepted, July 2003.

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0889-5406/2003/\$30.00 + 0

doi:10.1016/j.ajodo.2003.09.010