

# Release of nickel ions from stainless steel alloys used in dental braces and their patch test reactivity in nickel-sensitive individuals

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Nickel ions leached in sufficient quantities from nickel-containing alloys may induce nickel sensitization or elicit allergic contact dermatitis. Nickel-containing stainless steel alloys are generally considered safe for nickel-sensitive individuals to use. The study summarized in this paper investigated 3 parameters. First, the release of nickel was estimated in artificial saliva and sweat from 4 different stainless steel alloys frequently used in dental braces. Second, in a pilot study, oral mucosa cells harvested from 3 dental patients before and after the attachment of dental braces were analysed for possible nickel content. Third, patch test reactivity of the 4 stainless steel alloys was tested on 31 nickel-sensitive subjects. All 4 stainless steel alloys released small amounts of nickel ions into artificial saliva ( $<0.13 \mu\text{g}/\text{cm}^2/\text{week}$ ) and artificial sweat ( $<0.05 \mu\text{g}/\text{cm}^2/\text{week}$ ), but no measurable amounts of nickel were found in any of the oral mucosa samples. None of the 31 nickel-sensitive subjects reacted to patch testing with the 4 stainless steel alloys, indicating that these stainless steel alloys would be safe to use in direct and prolonged contact with the skin.

**Key words:** allergic contact dermatitis; dental braces; nickel release; patch test; stainless steel alloys.

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Nickel ions leached in sufficient quantities from nickel-containing alloys may induce nickel sensitization or elicit allergic contact dermatitis. Nickel-containing stainless steel alloys are generally considered safe for nickel-sensitive individuals to use. Stainless steel alloys used in the metallic parts of orthodontic braces contain 3–15.5 wg% nickel (1). The amounts of nickel ions released *in vitro* from dental braces depend upon the alloys and the test system employed (2–8). *In vitro* nickel ion release from nickel-chromium dental casting alloys (not stainless steel) has been reported as  $4.2 \mu\text{g}/\text{cm}^2/\text{D}$  in pooled human saliva (6), and *in vitro* release rate of full-mouth orthodontic appliances has been estimated to be in the range from  $26 \mu\text{g}$  nickel/D immersed in 0.05% sodium chloride to  $40 \mu\text{g}$  nickel/D immersed in artificial saliva (2, 4). Studies have

found nickel ion release from low-sulfur stainless steel alloys in artificial sweat to be less than  $0.03 \mu\text{g}/\text{cm}^2/\text{week}$ . Most stainless steel alloys release less than  $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ , but high sulfur stainless steel may release as much as  $1.5 \mu\text{g}/\text{cm}^2/\text{week}$  into artificial sweat (7, 8).

Saliva may potentially corrode the different alloys and thereby release nickel ions into the adjacent oral mucosa, but the quantity of the corrosive products that may be absorbed by dental patients is unknown. Therefore, the aim of the present study was to estimate the *in vitro* release of nickel ions into artificial saliva from 4 different stainless steel alloys frequently used in dental braces and to quantify the amount of *in vivo* release of nickel ions from dental braces by measuring the nickel content in oral mucosa cell samples taken adjacent to dental braces before and after attachment. Furthermore, the allergic reactivity of the same 4 stainless steel alloys was investigated by *in vitro* release testing in synthetic sweat and patch testing of nickel-sensitive subjects.

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## Materials and Methods

### *Leaching experiments of stainless steel alloys*

Leaching of nickel from 4 different stainless steel alloys of the austenitic stainless steel structure (DIN no. (European Standard designation number) 1·4303, DIN no. 1·4404, DIN no. 1·4435 and DIN no. 1·4541), frequently used in various parts of dental braces, such as brackets, bands, mesh pads and arches, was studied in two *in vitro* models. The chemical composition of the alloys is summarized in Table 1. Austenitic stainless steels, also known as the AISI (American Iron & Steel Industries designation number) 300 series, offer a greater resistance to corrosion because of their high nickel and chromium content.

From the 4 different stainless steel alloys, 20 discs from each alloy were cut out of metal sheets by laser. Surface areas of the stainless steel specimens exposed to artificial saliva or sweat solutions were as follows: DIN no. 1·4303: 1·055 cm<sup>2</sup>, DIN no. 1·4541: 1·055 cm<sup>2</sup>, DIN no. 1·4435: 1·105 cm<sup>2</sup> and DIN no. 1·4404: 1·105 cm<sup>2</sup>. Prior to the experiment, the discs were cleaned ultrasonically in a solution of 50% v/v acetone and 50% v/v ethanol. 10 discs from each of the 4 alloys were placed in 10 separate acid-rinsed polyethylene bottles (NUNC, Roskilde, Denmark) containing 5 ml artificial saliva (0·4 g NaCl, 1·21 g KCl, 0·78 g NaH<sub>2</sub>PO<sub>4</sub> · 2H<sub>2</sub>O, 0·005 g Na<sub>2</sub>S 9H<sub>2</sub>O, 1 g urea and 1000 ml distilled and then deionized water, pH 5·1) (4), and 10 discs from each alloy were placed in 10 similarly rinsed bottles containing 5 ml artificial sweat (5·0 g NaCl, 1·13 g lactic acid, 1 g urea and 1000 ml distilled and then deionized water, pH 6·5) (8). The bottles were placed in a Roto-Torque rotator (Cole-Palmer Instrument Co, Chicago, IL, USA) at 37° celsius for 1, 3, 7, 14 or 21D. After these time intervals, the discs were removed from the artificial saliva or sweat solutions and the nickel ion release was quantified by the use of electrothermal atomic absorption spectrometry (Perkin-Elmer Z-5100 or Perkin-Elmer SIMAA 6000; detection limit = 0·5 µg/l ~ 0·0025 µg/5 ml sample). Samples were tested in duplicate, and

duplicate contamination control samples of saliva and sweat without discs were analysed after 21D.

### *Pilot studies on nickel content in oral mucosa cells*

3 dental patients volunteered to participate in this pilot study. Oral mucosa cells from 2 of the patients were harvested for nickel content analysis before and 1 week after the attachment of dental braces. The implanted braces were made of different metallic alloys, with a nickel content ranging from 3·0 to 18·0 wg%. Additionally, 1 subject had 6 brackets made of stainless steel (DIN no. 1·4542; nickel content: 3·0–5·0%) implanted on the upper central incisors, lateral incisors and cuspids for 3 weeks. Oral mucosa cells from this patient were harvested for nickel content analysis before and in this case every 2nd day until 21D after the attachment. Before obtaining the oral mucosa cells, the volunteers washed their mouths 3 times in distilled and deionized water. By the use of a prerinsed polyethylene spatula, the superficial oral mucosa cells adjacent to the dental braces were scraped 3–4 times and transferred into acid-rinsed polyethylene bottles (NUNC) containing 5 ml distilled and deionized water. The samples were treated ultrasonically for 2–3 min, and 5 ml 0·1% HNO<sub>3</sub>/0·1% Triton X-100 in distilled and then deionized water was added to each sample. The samples were divided into two, and the nickel content in each sample was measured by the use of electrothermal atomic absorption spectrometry (Perkin-Elmer Z-5100 or Perkin-Elmer SIMAA 6000; detection limit = 0·5 µg/l ~ 0·0025 µg/5 ml sample). Duplicate contamination control samples were additionally analysed.

### *Patch tests*

Discs, 0·8 cm in diameter and 0·2–0·4 mm thick, of the 4 stainless steel alloys were used in a patch test using Scanpor tape<sup>®</sup> (Norgesplaster, Vennesla, Norway) and applied for 2D on the upper part of the back of 31 volunteering nickel-sensitive subjects who had recently been patch tested positive

Table 1. Chemical composition (wg%) of the stainless steel alloys used in the study

| DIN number* | AISI number† | Ni        | C     | Si   | Mn   | Cr        | Mo      | P      | S      | Others           | Fe      |
|-------------|--------------|-----------|-------|------|------|-----------|---------|--------|--------|------------------|---------|
| 1·4303      | 305          | 11·0–13·0 | <0·07 | <1·0 | <2·0 | 17·0–19·0 | None    | <0·045 | <0·03  | None             | Balance |
| 1·4541      | 321          | 9·0–12·0  | <0·08 | <1·0 | <2·0 | 17·0–19·0 | None    | <0·045 | <0·03  | Ti 5x% C bis 0·8 | Balance |
| 1·4404      | 316 L        | 11·0–14·0 | <0·03 | <1·0 | <2·0 | 16·5–18·5 | 2·0–2·5 | <0·045 | <0·03  | None             | Balance |
| 1·4435      | 316 L        | 12·5–15·0 | <0·03 | <1·0 | <2·0 | 17·0–18·5 | 2·5–3·0 | <0·045 | <0·025 | None             | Balance |

\*European Standard designation number.

†American Iron & Steel Industries designation number.

to nickel. Prior to the experiment, the discs were cleaned ultrasonically in a solution of 50% v/v acetone and 50% v/v ethanol. The test evaluations were performed at different time points 2 times within 5D after removal of the discs. Reactions were classified according to the International Contact Dermatitis Research Group recommendations (9).

Informed consent was obtained from all participating volunteers. The study was conducted with the approval of the Ethics Committee in Copenhagen County.

## Results

### Leaching experiments

During the study, no rust colouration of the artificial saliva or the artificial sweat was noticed in any of the samples or in any of the stainless steel discs. As seen in Table 2, all 4 stainless steel samples released detectable concentrations of nickel ions into the saliva (range: 0.005–0.152  $\mu\text{g}/\text{cm}^2$ ) and sweat (range: 0.007–0.059  $\mu\text{g}/\text{cm}^2$ ). It was noticed that although the alloys contained comparable amounts of nickel, the amount of nickel ions released into the saliva or sweat was not the same. The amounts of nickel ions released from each stainless steel alloy were found to be variable with time and not totally consistent. Additionally, the nickel ions released in the duplicate samples from some of the stainless steel alloys showed some variation between samples. Only the stainless steel alloy DIN no. 1.4303 and DIN no. 1.4404 showed an increase in the amount of nickel ions released into the saliva over time (DIN no. 1.4303 from 0.036 to 0.069  $\mu\text{g}/\text{cm}^2$  and DIN no. 1.4404 from 0.016 to 0.110  $\mu\text{g}/\text{cm}^2$ ), and most of the alloys released smaller amounts into the artificial sweat over time. The stainless steel alloy DIN no. 1.4541 released small amounts into both saliva (0.005–0.006  $\mu\text{g}/\text{cm}^2$ ) and sweat (0.007–0.018  $\mu\text{g}/\text{cm}^2$ ), whereas stain-

less steel alloy DIN no. 1.4435 on the contrary released the highest amount of nickel ions into the saliva after 1D (0.129  $\mu\text{g}/\text{cm}^2$ ), which barely accumulated in the following samples, and much lower amounts were leached into the sweat (0.017–0.041  $\mu\text{g}/\text{cm}^2$ ). The nickel contents of duplicate control samples of saliva and sweat without discs were below detection level (<0.5  $\mu\text{g}/\text{l}$ ).

### Pilot studies on nickel content in oral mucosa cells

We were not able to find measurable amounts of nickel in any of the mucosa samples from the 3 patients fitted with orthodontic braces. As no measurable amounts of nickel were found in any of the samples either before or after the attachment of dental braces, no effort was made to quantify sample sizes. The nickel contents of duplicate control samples were below detection level (<0.5  $\mu\text{g}/\text{l}$ ).

### Patch tests

None of the 31 nickel-sensitive subjects reacted to the patch tests with any of the stainless steel alloys.

## Discussion

Nickel ion release from stainless steel alloys has been demonstrated in many studies in various media (10). However, the majority of people previously sensitized to nickel are unlikely to elicit an allergic response to stainless steel alloys, when used in items that come into prolonged and close contact with the skin. Some studies suggest that orthodontic treatment with stainless steel braces does not initiate or aggravate an allergic reaction to nickel (11, 12), but there are rare case reports which show that some people may have a subsequent allergic response after the attachment of dental braces made of stainless steel (13–17).

Table 2. Nickel released in  $\mu\text{g}/\text{cm}^2$  from the tested stainless steel alloys in artificial saliva and in artificial sweat (mean value of duplicate samples and standard deviations (SDs))

| DIN number* | Mean after 1D (SD) | Mean after 3D (SD) | Mean after 7D (SD) | Mean after 14D (SD) | Mean after 21D (SD) |
|-------------|--------------------|--------------------|--------------------|---------------------|---------------------|
| In saliva   |                    |                    |                    |                     |                     |
| 1.4303      | 0.036 (0.0363)     | 0.011 (0.0054)     | 0.071 (0.0046)     | 0.052 (0.0059)      | 0.069 (0.0023)      |
| 1.4541      | 0.006 (0.0013)     | 0.006 (0.0006)     | 0.006 (0.0013)     | 0.006 (0.0002)      | 0.005 (0.0001)      |
| 1.4404      | 0.016 (0.0054)     | 0.031 (0.0032)     | 0.058 (0.0497)     | 0.052 (0.0059)      | 0.110 (0.0008)      |
| 1.4435      | 0.129 (0.0590)     | 0.122 (0.0579)     | 0.124 (0.0543)     | 0.124 (0.0563)      | 0.152 (0.0636)      |
| In sweat    |                    |                    |                    |                     |                     |
| 1.4303      | 0.027 (0.0088)     | 0.026 (0.0063)     | 0.040 (0.0020)     | 0.047 (0.0045)      | 0.047 (0.0043)      |
| 1.4541      | 0.008 (0.0004)     | 0.007 (0.0003)     | 0.018 (0.0046)     | 0.012 (0.0010)      | 0.014 (0.0032)      |
| 1.4404      | 0.034 (0.0136)     | 0.035 (0.0069)     | 0.045 (0.0030)     | 0.050 (0.0018)      | 0.059 (0.0009)      |
| 1.4435      | 0.017 (0.0004)     | 0.040 (0.0206)     | 0.041 (0.0176)     | 0.031 (0.0001)      | 0.038 (0.0023)      |

\*European Standard designation number.

In the present study, we were able to detect leaching of nickel ions in varying concentrations from 4 different stainless steel alloys frequently used in dental braces. Less than  $0.13 \mu\text{g Ni/cm}^2/\text{week}$  was found in the artificial saliva samples, and  $<0.05 \mu\text{g Ni/cm}^2/\text{week}$  was found in the synthetic sweat samples. Similar results have been found in other studies for synthetic sweat (7, 8). The variations in the nickel content found between duplicate samples and the slight inconsistencies found in the nickel leaching over time in some of the alloys are known from other studies (2–5).

We were not able to find measurable amounts of nickel in the oral mucosa of patients treated with orthodontic braces, and therefore, we have not been able to conclude that there is any absorption of nickel by the oral mucosa. The lack of measurable amounts of nickel in all samples may be because of the fact that concentrations of nickel in the samples are below the detection limit of our assay system ( $<0.5 \mu\text{g/l} \sim 2.5 \text{ ng/5 ml}$  sample). However, this does not exclude the possibility of *in vivo* corrosion and nickel ion release from dental braces and subsequent absorption by the oral mucosa adjacent to the braces. First of all, the mucosa cells obtained from the dental patients were only scraped off superficially so that the patients would not be hurt. This could have resulted in too small amounts of oral mucosa cells containing too low concentrations of nickel for our assay system to detect. Secondly, a quick transport of nickel ions out of the mucosa to the regional lymph nodes could result in only minor accumulation of nickel in the mucosa cells, explaining the lack of measurable amounts of nickel in the samples. Parameters that may influence the degree of nickel corrosion and nickel ion release from the dental braces and the subsequent absorption by the mucosa cells could be the differences in the individual's saliva production, the regional pH value in the surrounding area of the braces and the presence of proteins from food binding the released nickel.

Based on dermal elicitation threshold investigations using patch testing of nickel-sensitive individuals (18, 19), a nickel ion release threshold of  $0.5 \mu\text{g/cm}^2/\text{week}$  in synthetic sweat from nickel-containing alloys and coatings has been determined. This nickel release rate is the threshold used in the EU Nickel Directive (20) for items, which are intended for direct and prolonged contact with the skin. If exposure is to items that release less nickel than this nickel release threshold, only a small number of nickel-sensitive persons will have an inflammatory reaction (19).

Our finding that none of the nickel-sensitive patients reacted to any of the stainless steel alloys tested, which were found to leach  $<0.05 \mu\text{g/cm}^2/\text{week}$  in artificial sweat, indicates that stainless steel alloys leaching nickel ions at this level may be considered safe for nickel-sensitive individuals to use in direct and prolonged contact with the skin.

In conclusion, we found that the 4 different kinds of stainless steel alloys released small amounts of nickel ions into both artificial saliva ( $<0.13 \mu\text{g Ni/cm}^2/\text{week}$ ) and synthetic sweat ( $<0.05 \mu\text{g Ni/cm}^2/\text{week}$ ). However, no detectable nickel levels were found in oral mucosa samples, and none of the stainless steel alloys elicited patch test responses in any of the nickel-sensitive volunteers. Therefore, we are not able to state whether nickel is absorbed by the oral mucosa adjacent to the dental braces or to quantify the amount of nickel ions released from dental braces. The results of the patch testing of the stainless steel alloys leaching nickel ions at this level suggests that they may be considered safe for most nickel-sensitive individuals to use in direct and prolonged contact with the skin.

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