Tissue reactions to implanted orthodontic wires in rabbits

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Tissue response to a Fe-Cr-Ni and a Co-Cr-Ni orthodontic wire, in the as-received state and with silver soldered joints, was investigated. Specimens with polytetrafluoroethylene (PTFE) as a reference material were implanted in the subcutaneous tissue of rabbits. Six rabbits were sensitized to nickel, four animals were sham-sensitized, and two were left untreated. The results showed that the as-received wires gave no tissue response relative to the PTFE control. In soldered specimens there were moderate to extreme reactions adjacent to the soldered joint and around the wire portion as well. The soldered Co-Cr-Ni wire elicited the most severe reactions, most pronounced in the nickel-sensitized animals. The agar overlay cell culture test of some retrieved implants showed pronounced cytotoxicity of the soldered specimens. Leachable toxic components of the silver solder seemed to be of major importance in the observed cell culture and tissue response to the soldered specimens. □ *Cobalt-chromium alloy; metal hypersensitivity; silver soldering; stainless steel*

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Orthodontic wires are in close contact with the mucosa, gingiva, and saliva. Extraoral appliances may contact the skin as well. In spite of the temporary nature of orthodontic treatment, the materials may be in use for periods for up to 2 or 3 years. In general, the materials used in orthodontic treatment seem to be well tolerated, and few cases of adverse reactions have been recorded. However, several of the cases in which adverse reactions have occurred appear to be related to hypersensitivity reactions towards nickel.

Hypersensitivity to nickel-containing alloys may present a problem with orthopedic implants (1) and with alloys used in orthodontics, both in intraoral and extraoral appliances (2–4). Reactions to stainless steel wires have been reported in conjunction with maxillofacial surgery (5, 6).

Orthodontic alloys and appliances corrode and release nickel and other constituents (7–9). Soldering of orthodontic appliances enhances the release of metals (10, 11), and skin reaction to a soldered appliance has been reported (12).

Systematic information on the biologic properties of orthodontic alloys is sparse. In accordance with the results of suggested biologic tests, the material should be subjected to initial, low-level tests, such as cell culture and implantation tests (13).

Implantation tests have been applied to nickel-containing, surgical alloys, both in normal and nickel-sensitized animals. The implants may give rise to immunologic responses, as shown by macrophage migration inhibition tests in rabbits (14) and by patch testing in guinea pigs (15).

The aim of this study was to evaluate the tissue reaction to two nickel-containing orthodontic wires, with and without silver soldered joints. The specimens were implanted in the connective tissue in rabbits with and without enhanced sensitivity to nickel. Additionally, the cytotoxicity of some of the retrieved implants was assessed in a cell culture test.

Materials and methods

Alloys

A stainless steel (Fe-Cr-Ni) wire and a cobalt-chromium (Co-Cr-Ni) wire with diameters of 1.05 to 1.12 mm, respectively, were used (Table 1).

Table 1. The products used in the investigation and their approximate chemical composition

Material	Product	Manufacturer	Batch no.	Approx. composition (weight %)
Fe-Cr-Ni wire	Tru-Chrome	Rocky Mountain/ Orthodontics, Denver, USA	2516	Fe 68, Cr 19, Ni 13*
Co-Cr-Ni wire	Elgiloy Soft	Rocky Mountain/ Orthodontics, Denver, USA	6843	Co 40, Cr 20, Ni 15, Fe 15†
Silver solder	Orthodontic silver solder	Unitek Corp., Monrovia, USA	—	Ag 60, Cu 22, Zn 18*

* Analyzed by E. S. Erichsen, School of Dentistry, Bergen, Norway.
† From: Compilation of chemical compositions and rupture strengths of superalloys. ASTM, 1968.

One set of specimens consisted of the as-received wire; another set was made from the same type of wire, which included a silver soldered joint (Fig. 1). The soldered specimens were made by cutting the wire with carborundum discs. The pieces were realigned in a fixture; borax flux and about 0.05 g of silver solder were applied and heated with a propane torch for 3–5 sec until the solder flowed freely and then cooled in air. The specimen was discarded if the soldering was not immediately successful. After being soldered, the specimens were cleaned in water and ethanol in an ultrasonic cleaner, to remove the flux. As a reference material a length of about 6 mm of heat-shrinkable polytetrafluoroethylene (PTFE) tubing was applied around one end of each specimen (Fig. 1). The tubing was shrunk at approximately 250 °C for a few seconds, until a tight fit was obtained.

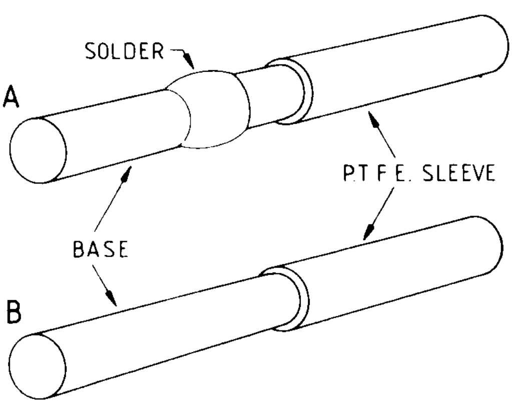

Fig. 1. The specimens used in the investigation. (A) soldered specimen; (B) specimen made from as-received wire. The PTFE sleeve served as a reference material in the histologic evaluation. The diameter of the wires was approximately 1.1 mm, and the length 16 mm.

Animals

Twelve Chinchilla rabbits, nine female and three male, weighing between 2.9 and 3.7 kg (mean, 3.3 kg), were used in the investigation. The rabbits were caged separately and fed a pelleted diet and water ad libitum.

The animals were weighed regularly, and changes in weight between the different groups of animals were compared by the Kruskal–Wallis test for independence at a significance level of 5%.

Six rabbits received intramuscular injections of 0.5 ml 2% $NiCl_2$ in 0.85% NaCl, suspended in an equal volume of Freund's complete adjuvant (FCA) (Behringwerke AG, Marburg, FRG). Two booster injections of 2% $NiCl_2$ in 0.85% NaCl were given at 3 weeks' interval ('sensitized' animals). Four rabbits received one injection of FCA in 0.85% NaCl, followed by injections of 0.85% NaCl, in accordance with the same time protocol as described above ('sham-sensitized' animals). Two animals received neither FCA nor nickel injections ('untreated' animals). The purpose of the FCA was to enhance the production of antibody in the animals.

Nickel sensitivity was evaluated 4 weeks after start of sensitization by intradermal injections in the shoulder region of 0.02% and 0.2% $NiCl_2$ and solvent with no nickel. The reactions were read after 4, 24, 48, 96, and 120 h.

Implantation and histologic technique

Six weeks after the start of sensitization, one of each of the different types of specimens was implanted in each rabbit in the subcutaneous tissue of both flanks. The implants were left in place for 31 days. To minimize operational trauma, the specimens were implanted by passing them through a stainless steel tube, which was inserted through a central incision approximately 1 cm long. The wound was closed with two to three silk sutures.

When the animals were killed, the specimens and the surrounding tissue were excised and fixed in 10% neutral-buffered formalin. The tissue blocks were paraffin-embedded as for conventional histologic technique. The blocks were cut in a microtome until the implant could be removed with dissecting pins. The tissue specimens were then re-embedded before final sectioning. Serial sections, about 5 μm thick, were stained with hematoxylin–eosin and Masson trichrome stain to show general morphology.

Histologic evaluation was based on cell morphology, cellular density, and vascularity and was classified as no, slight, moderate, severe, and extreme reactions (16). The tissue reaction to the metal was evaluated relative to the reactions adjacent to the PTFE tubing.

Cell culture technique

The agar overlay technique (17) was used to evaluate the cytotoxity of substances leaching from some of the specimens that had been retrieved after implantation. Before being tested, they were successively cleaned in xylol, ethanol, and water in an ultrasonic bath. New PTFE tubing was then applied. Plastic petri dishes, 54 × 12 mm, were seeded with 10^6 cells (NCTC 2544) in 5 ml Eagle's minimum essential medium (MEM) (Flow Laboratories Ltd., Irvine, Scotland) with 10% calf serum. A confluent cell layer was formed after 2–3 days. The medium was then replaced by 2.5 ml Eagle's MEM with 2% oxoid agar and 1% calf serum. The cells were stained with neutral red. The specimens were placed on top of the agar and stored in an incubator (37 °C, 4% CO_2, 100% humidity) for 24 h. A piece of polyethylene tubing was included as a negative control in each dish. At the end of the incubation period the cells were fixed in 4% formalin, the implants and agar removed, and the cells stained with 2% crystal violet. The cytotoxic reaction was evaluated by the width of the inhibition zone.

Scanning electron microscopy and X-ray analysis

Retrieved implants were cleaned as described for the agar overlay test, coated with gold and examined in the secondary electron emission mode.

To show metal particles in the tissue, two paraffin blocks were subjected to electron microscopy by the back-scattered electron mode. A qualitative chemical analysis was performed by energy-dispersive X-ray spectroscopy.

Results

The weight of the animals increased 8% on average during the experiment. No rabbits lost weight, nor were there statistical differences in weight changes for the three experimental groups.

Challenge

The reading of the intradermal challenge indicated that four of six rabbits in the sensitized group and two of four animals in the sham-sensitized group had developed a hypersensitivity to nickel. The intradermal injections were placed in a shaved area in the shoulder/neck region, which turned out to be accessible for the rabbits' hind feet. Most of the reactions were difficult to assess because they were obscured by severe scratch marks.

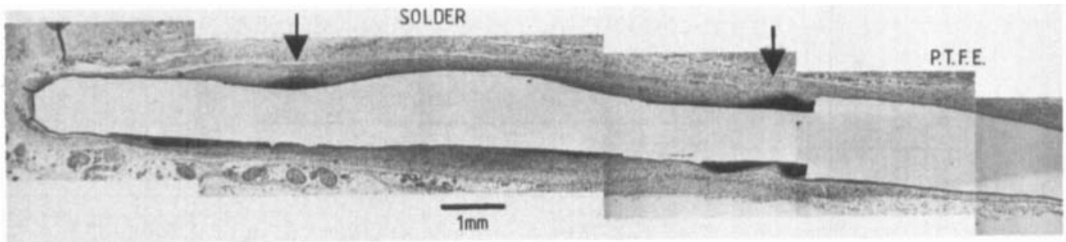

Fig. 2. Low-power micrograph showing the tissue reaction to a soldered Co-Cr-Ni wire in a nickel-sensitized rabbit. Reactions rated as 'extreme' are found at the metal/PTFE junction and at the left-hand portion of the wire (arrows). The reaction adjacent to the solder was rated 'severe'. See Fig. 1 for orientation of the specimen.

Histopathologic evaluation

Four implants were not recovered at death, and two tissue blocks were lost because of mishaps in the histologic processing.

An example of the low-power microscopic appearance of a histologic section is shown in Fig. 2. The results of the histopathologic evaluation are presented in Fig. 3. Enhanced tissue reaction to the as-received (unsoldered) wires compared with the PTFE tubing could not be detected for either alloy or in any group of animals. The specimens were surrounded by a thin capsule of mature collagen, as indicated by the Masson trichrome stain. The capsule was well organized and contained fibroblasts and fibrocytes.

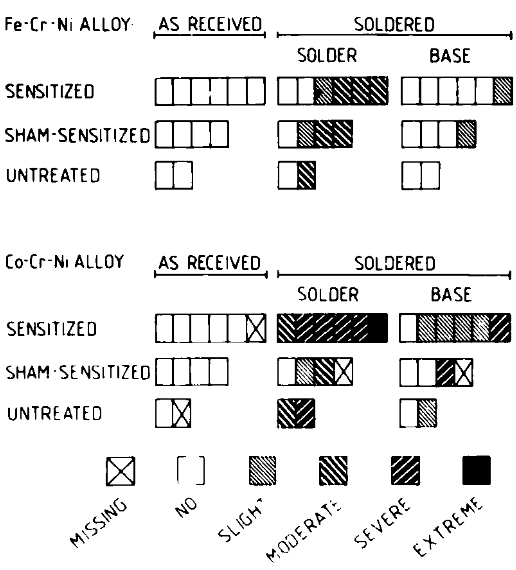

Fig. 3. The tissue reaction to different types of wires and different portions of the soldered specimens in the three groups of animals. 'As-received' refers to the unsoldered wires (see Fig. 1B), and 'soldered' refers to specimens with a soldered joint (see Fig. 1A). For the soldered specimens reactions to the solder and the base are scored separately. Each block represents one animal. The tissue response is scored relative to the reactions along the PTFE sleeve of each specimen.

The tissue reactions to the soldered Fe-Cr-Ni wire ranged from no to slight for both the wire portion and the soldered area. There were no apparent differences between the various groups of animals. The reaction to the wire portion of the soldered Co-Cr-Ni wire was somewhat greater than to the Fe-Cr-Ni wire. The reactions in the vicinity of the soldered area of the Co-Cr-Ni wires were more intense in the nickel-sensitized animals. The tissue reaction had the character of acute inflammation, with abundant polymorphonuclear cells and high vascularity. The capsule appeared to consist of immature collagen in the solder region.

In a few specimens macrophages in the vicinity of the soldered area appeared to contain small particles. X-ray analyses of the same region in two of the paraffin-embedded tissue blocks indicated the presence of three types of particles. They consisted of iron + chromium + nickel, of copper + zinc, or of silver. The X-ray analyses of the paraffin blocks did not show soft-tissue structures, making it difficult to localize the particles exactly.

The cell culture test showed 20- to 25-mm-wide zones of cell lysis around the soldered specimens. The width of the reaction zone around the soldered Co-Cr-Ni wire was

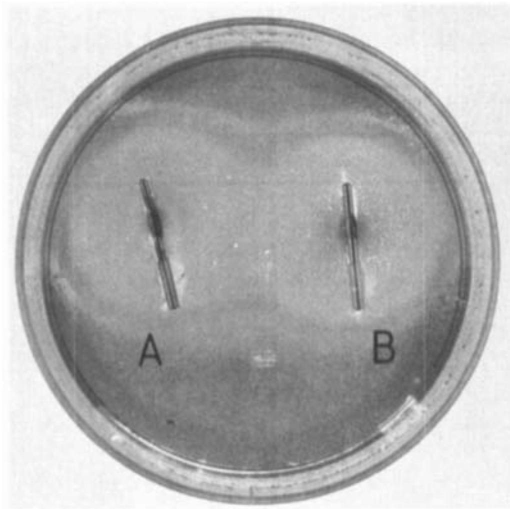

Fig. 4. Agar overlay cell culture test of retrieved implants with soldered joints. (4A) Fe-Cr-Ni wire; (4B) Co-Cr-Ni wire.

similar to the one around the Fe-Cr-Ni wire (Fig. 4). Close to the solder there was a brownish discolored area. There were no observable reaction zones around any part of the specimens made from as-received wires (Fig. 5).

Scanning electron microscopy of some of

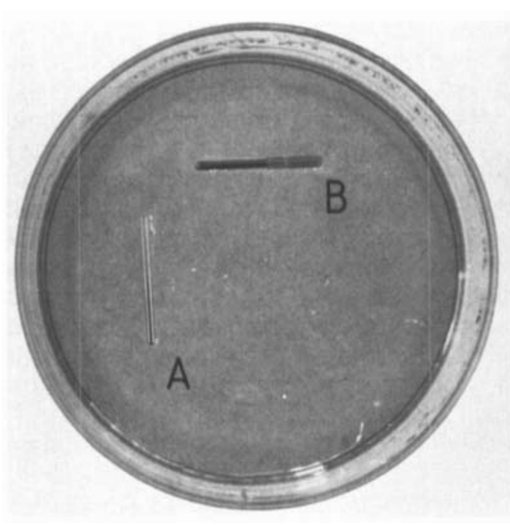

Fig. 5. Agar overlay cell culture test of retrieved implants consisting of as-received orthodontic wires. (A): Fe-Cr-Ni wire; (B): Co-Cr-Ni wire.

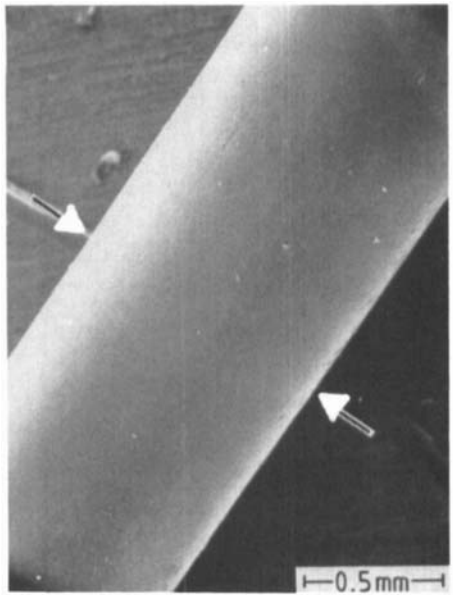

Fig. 6. Scanning electron picture of retrieved Fe-Cr-Ni implant. PTFE tubing has covered the lower part of the specimen, to a level indicated by the arrows on each side of the wire.

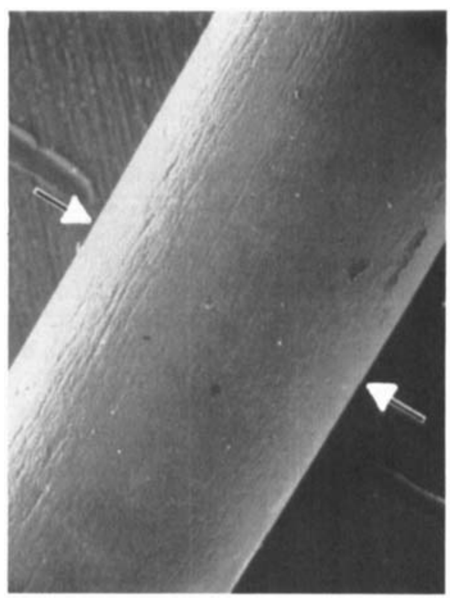

Fig. 7. Scanning electron picture of retrieved Co-Cr-Ni implant. See also legend to Fig. 6. The magnification is the same as in Fig. 6.

the retrieved implants showed pitted areas on the solder. No signs of crevice corrosion at the metal/PTFE interface or corrosion attacks of the exposed base were observed. The Co-Cr-Ni wire had a somewhat rough surface with longitudinal grooves compared with the Fe-Cr-Ni wire, which had a uniform, smooth surface (Figs. 6 and 7). The appearance was not different from that of unused implants.

Discussion

The present study could not demonstrate enhanced tissue reaction to the two types of as-received nickel-containing wires tested, even in animals with presumably increased sensitivity to nickel. Soldered specimens, however, gave pronounced reactions, particularly the soldered Co-Cr-Ni specimens in nickel-sensitized animals. Orthodontic solders contain potential irritants, particularly copper and zinc, which are released in considerable amounts as revealed by in vitro tests (10, 11). Both elements exert strong cytotoxicity in cell culture tests (18). Casting alloys with up to 84% nickel are not cytotoxic in cell culture tests (19). The more severe reactions found around the silver soldered Co-Cr-Ni implants used in this study are in accordance with in vitro findings showing more release of copper after 24 days from a soldered Co-Cr-Ni wire than from a soldered Fe-Cr-Ni type (11).

The dense infiltration of inflammatory cells adjacent to the soldered joints is similar to the reaction found around a Pd-Cu-Ag alloy implanted in guinea pigs (20). Furthermore, it resembles the reaction that has been described in intramuscular implants of pure copper (21). The release of toxic particles from the soldered areas may have contributed to the total tissue reaction. However, the present methods did not allow firm conclusions as to when the particles were introduced into the tissue. The reaction could have been due to release of particles in vivo but also to contamination of the tissue block or the sectioning blade during sectioning with the implants in situ.

The results of the agar overlay test confirmed the toxic nature of the soldered joints and the benign behavior of the as-received wires. The stained zone close to the solder was probably due to high local concentration of copper, which had fixed the cells in situ, as described for a copper-containing casting alloy (20).

The tissue reactions found along the wire portion of the soldered specimens may have been due to the reduced corrosion resistance caused by the heating necessary to melt the silver solder (22). It is, however, more likely that toxic substances leaching from the solder had spread along the surface of the wire. It can be speculated that the diffusion takes place more readily along the Co-Cr-Ni wire because of its rougher surface texture comprising longitudinal grooves (Fig. 7). A study of surgical Co-Cr alloys indicates that the roughness as such is of minor importance as to tissue reactions (23). Remnants of the borax flux used during soldering could also have caused reactions. The specimens were thoroughly cleaned before implantation, with this possibility in mind.

The reaction found at the junction between the wire and the polymer sleeve in the Co-Cr-Ni specimens may indicate that crevice corrosion had taken place at the interface between the PTFE tubing and the wire. However, scanning electron microscopy of retrieved implants could not show corrosion defects in this region.

The present study did not demonstrate distinct differences in tissue reaction in the nickel-sensitized animals compared with the sham-sensitized ones. The results of the intradermal challenge testing were not conclusive as to nickel sensitivity. It has been shown that guinea pigs may develop immunologic tolerance owing to nickel and chromium in the diet (24). This may be a factor to consider in the present study, as the animals were housed in metal rib cages, and their history of elemental intake is unknown.

The soldered Co-Cr-Ni wire showed proportionally more severe and moderate reactions than the soldered Fe-Cr-Ni wires. However, the two untreated animals showed one moderate and one severe reaction to the same type of specimen. The release of nickel may be too low to elicit reactions, even in sensitized animals. Stainless steel and cobalt-chromium wires show a high degree of electrochemical passivity (25), and they most probably release only minute amounts of metals. Furthermore, the present test situation excludes mechanical factors, such as loading and metal-to-metal contact, which will tend to increase corrosion in a clinical situation.

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