Corrosion of dental alloys studied by implantation and nuclear tracer technique

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Brune, D., Kjærheim, Å., Hensten-Pettersen, A. & Marion, L. Corrosion of dental alloys studied by implantation and nuclear tracer technique. *Acta Odontol. Scand.* 1983, 41, 129–134. Oslo. ISSN 0001-6357.

A method for the study of in vivo corrosion of various dental materials such as amalgams, gold, and chromium–cobalt alloys combining implantation and nuclear tracer techniques is described. Radioactive and nonradioactive implants were inserted subcutaneously in the backs of two groups of rats. After 5 days all animals were killed. The adjacent tissue was submitted to histopathological examination. The animals exposed to radioactive amalgams and chromium–cobalt disks with activity levels of 2–4 mCi showed local tissue reactions identical to those seen in sham-operated animals—that is, inflammation and formation of granulation tissue. In contrast, the rats exposed to radioactive gold foils with activity levels of about 20 mCi suffered heavier damage at the implantation site, with loss of hair and some necrosis, in addition to the inflammation and granulation tissue formation. There was no kidney damage. The release of mercury, gold, or cobalt into the adjacent tissue and the kidney was measured by means of gamma-ray spectrometry. □ *Dental materials; implantation test; radiation injuries; tracer technique*

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The major biological assessment programs for dental materials are at present ADA and FDI's 'Recommended Standard Practices for Biological Evaluation of Dental Materials' (22) and BSI's 'Methods of Biological Assessment of Dental Materials' (21). Considered generally, all proposed standards comprise three levels of dental materials testing. On the first level there are several initial, or screening, tests for general toxicity of dental materials. On the second level the local toxicity of dental materials is evaluated. A typical representative on this level is the implantation test. Usage tests on laboratory animals represent the third level.

The proposed schedules for biological testing of dental materials suggest methods for each testing level. For the acceptance of a new dental material only correlation of results from different levels is of use. In practice, however, contradictory results are often obtained, even with old and clinically acceptable materials. This indicates that some of the proposed test methods may not be entirely appropriate. The proposed procedures, for example, do not emphasize the correlation of results from different test levels. All documents emphasize the need for revision of biological testing programs as more information becomes available. A set of specific tests at all three levels should be proposed for each group of dental materials—that is, one test level could entail that different test methods are performed for each group of dental materials.

For the assessment of dental alloys the biological corrosion study should be of prime importance, but it has not yet been included in the recommended standards. Corrosion studies of dental alloys are usually performed by in vitro experiments as a part of physical/chemical testing procedures of dental materials. Only a few biological corrosion studies of dental alloys are described in the literature (2–4, 9, 16, 19). Before a dental material is used clinically, its biological and physicochemical characteristics should be known (14). With regard to dental alloys this

Table 1. Dental alloys studied

Type	Alloy	Manufacturer
Amalgam	Revalloy®	S.S. White Ltd., Middlesex, UK
Amalgam	Dispersalloy®	Johnson & Johnson, Dental Products Co., N.J., USA
Gold	Degulor M®	Degussa, Pforzheim, FRG
Chromium–cobalt	Wironit®	Bego, Bremer Goldschlägeri, Wilh. Herbst, Bremen, FRG

means also including biological in vitro and in vivo corrosion testing as a part of the biological assessment of alloys.

The present methodological study shows the feasibility of accomplishing an in vivo corrosion study of dental alloys by combining nuclear tracer and subcutaneous implantation techniques, thus giving the implantation test a new use. The combined technique involves the subcutaneous implantation of a radioactive specimen that is exposed to the body fluids. Specific ions are released from the metal surface and deposited in the adjacent tissue and are subsequently transported to and deposited in various organs or eliminated from the body through excretion (4, 9). Various radionuclides inherent in dental alloys have half-lives that range from a few hours to several days. Consequently, the experiment must in such cases be completed within a period of time from 1 to 10 days. However, with long-lived nuclides the study can be considerably prolonged.

Injuries arising from absorption of β-particles and low energy γ-radiation may develop in the tissue adjacent to the operated region. Histopathological examination of the adjacent tissue was performed in the present study to evaluate whether the radiation doses submitted to this tissue could essentially change the experimental conditions and possibly influence the corrosion rate.

Materials and methods

Various alloys were exposed to neutron irradiation in a nuclear reactor. Standards—that is, known amounts of the elements studied—were irradiated simultaneously under the same conditions for the purpose of comparison. The resulting radioactive alloys were inserted in the animals subcutaneously. The implants were removed after a defined time of exposure. Tissues and organs were dissected and submitted to histopathological examination or radioactivity measurements.

Materials

The various dental alloys used in the present study are described in Table 1. The amalgam and gold specimens were $5 \times 10\ mm^2$, whereas the chromium–cobalt samples measured $10 \times 10\ mm^2$. The amalgam and chromium–cobalt alloys were 0.4 mm thick, whereas the gold specimens had a thickness of about 0.05 mm. The samples were ground with 1000 grit paper to obtain comparable surface characteristics.

Irradiation

The amalgam and chromium–cobalt specimens were irradiated for periods of 1 h and 5 h, respectively, in a thermal neutron flux of $5 \times 10^{12}\ n \cdot cm^{-2} \cdot s^{-1}$ in the nuclear reactor at Kjeller, Norway, resulting in activity levels of 2–4 mCi. The gold specimens were irradiated about 1 h in the same flux, attaining activity levels of about 20 mCi (18). This high activity was necessary owing to the greater corrosion resistance of gold alloys.

Subcutaneous implantation

Twelve male Wistar rats, weighing 200–250 g, were anesthetized with pentobarbital sodium (Nembutal®). The metal foils were inserted through incisions at the dorsal surface after blunt dissection of the interface of the subcutaneous and muscle tissue. The wounds were closed with metal clips. Radioactive foils or disks were implanted in seven animals in a hood shielded with lead and equipped with a lead glass window. Non-radioactive implants were inserted in five animals in a control group. Three of the control animals were sham-operated on the opposite side of the back, omitting the foils.

Histopathological examination

Five days after implantation all rats were killed in a carbon dioxide atmosphere, and the wound area and kidneys were removed for histological examination. The tissues were fixed in 4% formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin.

Radioactivity measurements

Tissue adjacent to the implant, comprising 1–2 g, and both kidneys were measured by gamma-spectrometry using a 7.6 × 7.6 cm NaI(Tl) crystal or a Ge(Li) semiconductor detector connected to multichannel analyzers. The following nuclides were measured in the various tissues: ^{197}Hg ($t_{\frac{1}{2}} = 65$ h), ^{203}Hg ($t_{\frac{1}{2}} = 47$ days), ^{198}Au ($t_{\frac{1}{2}} = 2.7$ days), ^{60}Co ($t_{\frac{1}{2}} = 5.2$ years). Mercury, gold, and cobalt were assayed quantitatively by comparing the activities in samples with those of the standards.

Results

In all animals, irrespective of treatment, the local tissue reaction was acute or subacute inflammation and repair in the form of production of granulation tissue (Fig. 1). There was no qualitative difference between the experimental groups in this respect, except that the radioactive gold implants caused loss of hair and some necrosis of tissue in addition

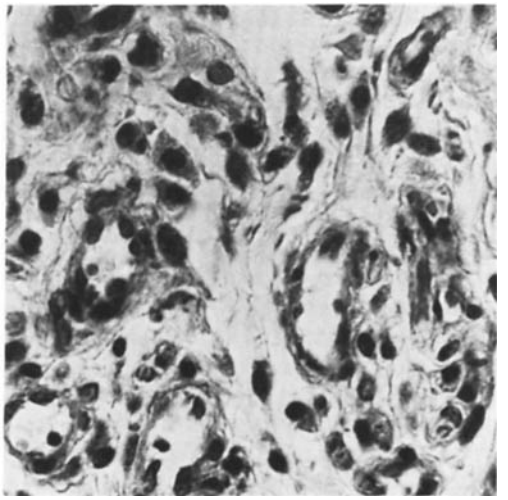

Fig. 1. Micrograph from the back of a rat that had been sham-operated on, without insertion of metal foil. Granulation tissue with newly formed vessels and acute and chronic inflammatory cells, 5 days after operation. There is no necrosis. (×500.)

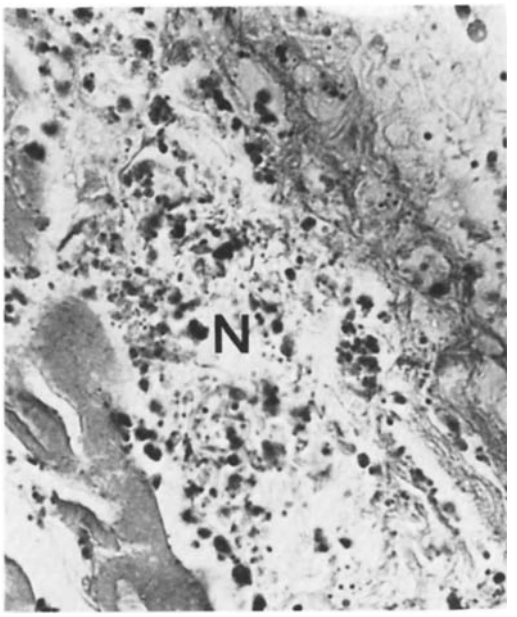

Fig. 2. Micrograph from the back of a rat in which a radioactive gold foil had been implanted subcutaneously 5 days previously. Necrosis (N) of connective tissue adjacent to the muscle fibers and loss of hair over the implantation site. (×500.)

Table 2. Release of mercury, gold, or cobalt from dental alloys implanted in seven rats. Each rat had one implant

Type	Alloy	Element	No. of rats	Amount, μg/g tissue	
				Adjacent tissue	Kidney
Amalgam	Revalloy®	Mercury	1	20	0.8
	Dispersally®	Mercury	1	19	1
Gold	Degulor M®	Gold	2	0.66, 0.95	0.004, 0.009
Chromium–cobalt alloy	Wironit®	Cobalt	3	0.36, 0.14, 0.18	

to the inflammatory reaction (Fig. 2). There was no kidney damage.

The amounts of mercury, gold, and cobalt measured in the adjacent tissue or kidney are presented in Table 2.

Discussion

Corrosion studies in vivo

Biological corrosion testing should be an integral part of the biological evaluation of dental alloys and be included in future biological assessment programs. The method presented could be performed as a first- and second-level test in the set of tests for the biological assessment of dental alloys. Finding and tracing the corrosion products from dental alloys can be of value both from the toxicological and allergenic point of view. The possibility to choose alloys, proven by biological testing not to corrode, might be of interest for patients with metal allergies. The standard implantation test, which is generally performed for screening toxicity properties of dental materials (11, 13, 14, 22), can be combined with corrosion studies. In that case, flat implants must be substituted for the rod-shaped ones to obtain high surface to volume ratio leading to lowest possible activity in the implant.

Different in vivo methods for investigating corrosion have been used previously. Histological evaluation of tarnish film and dyes in tissue adjacent to the implants has been studied after a 30-month observation period (16). This study was solely qualitative. The implantation technique has been used in mice to trace nickel released in vivo by atomic absorption spectrophotometry after a 5- or 6-month observation period (2, 4). The implantation and nuclear tracer technique provides the possibility to study release of corrosion products, to trace them, and to locate target organs and tissues.

Histopathology

During the observation period the animals were subjected to three different traumata: the surgical incision, the presence of a foreign body in the tissue, and exposure to radiation. The surgical incision alone caused an intense local reaction in the form of inflammation and production of granulation tissue as part of the repair process. The presence of inactive metal foils did not seem to add substantially to the tissue reaction—that is, no foreign body reaction in the form of giant cells was observed. This may have been due to the fact that there was, as compared with other types of foreign bodies, very little dissolution of metal ions in these experiments. This observation fits well with the situation when amalgam has been accidentally deposited in the oral mucosa after tooth extractions. Such amalgam deposits generally cause little tissue reaction (17).

The effect of radioactive exposure was in most cases obscured by the already heavy tissue reaction to the surgical procedure. The reaction was more severe than after the surgical incision alone only with the highest doses of radioactivity in the gold foils.

As expected, the biological situation in the tissue was not normal in these short-term experiments. Because of the acidity of the tissue fluids during acute inflammation (15),

it is reasonable to assume that the corrosion rate of metallic implants would be lower in long-term experiments, when the pH of the tissue fluids returns to normal levels.

Release

It is interesting to note in connection with the dissolution of gold from the implants, that organic gold salts are used in the treatment of rheumatoid arthritis. Administered as intramuscular injections to patients, the aim is to obtain a concentration of about 0.5 mg gold per 100 ml serum. This is a much higher concentration than was obtained in our experiments. For comparison, only about 1 μg gold was deposited in the adjacent tissues after 5 days in our experiment. This is probably less than the daily intake of gold in food and beverages, which has been estimated to be up to 7 μg/day (8).

The release of mercury, gold, or cobalt from the various dental materials (Table 2) represents a pilot study. A comprehensive investigation of the release of corrosion products in vivo from various dental alloys measured by the technique described will be presented elsewhere (19).

In the present study the nuclear tracer technique was chosen as a method to measure the in vivo corrosion rates of the specific elements from various dental alloys. This technique offers three main advantages: the analytical technique is sensitive, the analysis is accomplished without interference, and it is not necessary to know the normal distribution of the element studied in a specific tissue. The choice of a nonnuclear method for corrosion study would require that each tissue is analyzed carefully with regard to the normal distribution of the element to be measured. The in vitro corrosion of radioactive implants in saline solutions has been studied by a similar technique with amalgam, gold alloy, and steel samples (5–7).

Radiation doses

The present study was aimed at assessing corrosion of dental alloys in the biological environment. Consequently, tissue interaction close to the foils or disks was of main interest—that is, possible reactions in the adjacent tissue. This tissue would be affected by absorption of β-particles possessing small penetration depth, whereas the emitted γ-radiation would mainly be of importance for the whole-body dose. The radiation doses submitted to the adjacent tissues from the various implants were roughly estimated as follows.

The β-particles emitted from the radioactive specimens were assumed to be absorbed in 1 g of adjacent tissue. The strongest β-emitter in the present study was ^{198}Au, with β max = 0.96 MeV (10). Assuming one third of the emmitted β max energy to be absorbed in the tissue according to the shape of the β-spectrum (12), a dose of the order of 10,000 rad/mCi/day would be submitted to the tissue adjacent to the gold foils. Considering a β-mean energy from the nuclides ^{51}Cr, ^{64}Cu, ^{110m}Ag, ^{197}Hg, or ^{203}Hg in the amalgam or chromium–cobalt alloys of 0.1 MeV (10), a dose of about 3000 rad/mCi/day would be deposited in 1 g tissue. A large part of the β-energy would, however, be absorbed in less than 1 g of tissue surrounding the specimens, resulting in high local doses, since a major part of the β-particles would be absorbed to a depth of about 1 mm of the tissue (1).

In this context it should also be emphasized that self absorption of β-particles in the radioactive specimens occurs, resulting in lower doses submitted to the adjacent tissue. Thus, from the 0.4-mm-thick amalgam and chromium–cobalt alloy specimens less than 10% of the β-particles would be emitted to the tissue due to self absorption. The penetration depth of the particles in these materials would be of the order of 20 μm (4). On the other hand, a large part of the β-particles emitted from ^{198}Au in the gold foils would be absorbed in the tissue, considering β-particle penetration depth of the order of 100 μm (4) in the 50-μm-thick gold specimens.

The animals in the present study were submitted to high radiation doses. It does not seem advisable to exceed the activity level of 1 mCi for an exposure period of about 5 days, considering possible radiation injuries. On the other hand, it might be

difficult to perform corrosion studies of gold foils with lower activity levels in view of the high corrosion resistance of this material under the present conditions. One way to overcome such difficulties is to prepare very thin foils to obtain a high specific activity on the surface of the alloy. It should be mentioned in this context that doses of up to 6000–7000 rad are given to localized tumors in cancer treatment, whereas doses in thyroid or kidney scan using radiopharmaceuticals may attain values of up to 50 rad (20). High radiation doses may also shorten the life span. After whole-body exposure of mice to 600 rad using X-radiation, an average survival time of 43 weeks was recorded, as compared with 69 weeks for a non-irradiated control group (20). Deaths due to radiation injuries were not observed in the present investigation.

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Received for publication 14 December 1981