

Titanium: a review on exposure, release, penetration, allergy, epidemiology, and clinical reactivity

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Summary

Exposure to titanium (Ti) from both implants and from personal care products as nanoparticles (NPs) is common. This article reviews exposure sources, ion release, skin penetration, allergenic effects, and diagnostic possibilities. We conclude that human exposure to Ti mainly derives from dental and medical implants, personal care products, and foods. Despite being considered to be highly biocompatible relative to other metals, Ti is released in the presence of biological fluids and tissue, especially under certain circumstances, which seem to be more likely with regard to dental implants. Although most of the studies reviewed have important limitations, Ti seems not to penetrate a competent skin barrier, either as pure Ti, alloy, or as Ti oxide NPs. However, there are some indications of Ti penetration through the oral mucosa. We conclude that patch testing with the available Ti preparations for detection of type IV hypersensitivity is currently inadequate for Ti. Although several other methods for contact allergy detection have been suggested, including lymphocyte stimulation tests, none has yet been generally accepted, and the diagnosis of Ti allergy is therefore still based primarily on clinical evaluation. Reports on clinical allergy and adverse events have rarely been published. Whether this is because of unawareness of possible adverse reactions to this specific metal, difficulties in detection methods, or the metal actually being relatively safe to use, is still unresolved.

Key words: contact allergy; corrosion; dermatitis; hypersensitivity; lymphocyte transformation test; patch test; penetration; release; titanium; toxicity.

Titanium (Ti) is a non-essential metallic element with the atomic number 22 and a silvery colour. It is the ninth most abundant element in the earth's crust and the seventh most abundant metal overall (1). Major deposits of Ti minerals are found in Australia, Canada, India, Norway, South Africa, Ukraine, and the United States. The +4 oxidation state dominates Ti chemistry, but compounds in the +3 oxidation state are also common. The

most important oxide is Ti dioxide (TiO₂), which exists in three different crystalline forms – anatase, brookite, and rutile – in addition to an amorphous phase (2, 3). Both the anatase, rutile and brookite mineral forms of TiO₂ occur naturally.

TiO₂ is widely applied as microparticles and nanoparticles (NPs) in consumer goods, including cosmetics and foods, to obtain a white colour and ultraviolet light protection (4). Moreover, Ti is combined with a variety of other elements to produce strong lightweight alloys that provide resistance against corrosion and show a very high strength/density ratio (5). Because of this and the excellent biocompatibility of Ti, Ti alloys and commercially pure Ti (cpTi) have been widely used as alternatives to other metals in invasive medicine, surgery and dentistry during the last three decades (6).

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The existence and risk of allergy to Ti and TiO₂ have been much debated (7). The reason for this discussion is the increase in the number of case reports describing adverse reactions to Ti-based alloys and the extremely low rates of positive patch test reactions to Ti salts, especially TiO₂. With the substantial increase in the use of TiO₂ in personal care products and in Ti-based medical and dental implants, there is a continuous need to evaluate the safety of Ti in terms of sensitization and clinical reactivity. This review article provides an update on Ti allergy.

Materials and Methods

This is a non-systematic review. We searched the database PubMed MEDLINE up to November 2015, using the following search terms: allergy, corrosion, dental, dermatitis, hypersensitivity, implantation, lymphocyte transformation test, patch test, penetration, release, sensitization, titanium, and toxicity. The keywords were applied to the database in different combinations, and no limits with regard to time period or type of work were utilized. Additionally, the reference lists of relevant articles were searched for further studies.

Sources of Ti Exposure

Approximately 95% of Ti used globally is not in its metallic form, but is in the form of TiO₂ (8), a pigment valued for its chemical stability, brightness, ultraviolet light resistance, and presumed non-toxicity (4, 5, 9). The physical properties of Ti also make it a key material for use in airframes, jet engines, spacecraft, missiles, sports equipment, and implants (5) (Fig. 1).

Implantation

Ti is the material of choice in reconstructive (plates and screws) and cardiovascular (pacemaker encasing and

cardiovascular stents) surgery. Ti and its alloys are also widely used for orthopaedic implantation (joint replacement) (6, 10), because of the high biocompatibility and strength/density ratio of Ti. Ti and its alloys are also standard materials for dental implants (endosseous implants). For orthodontic appliances, which are fixed to the teeth and are not in contact with blood or bone, stainless steels and nickel–Ti are widely used. For dental crowns and bridges, which are also fixed to the teeth, an enormous variety of alloys, including Ti alloys, are used.

The alloy Ti–6Al–4V, which was originally used for aerospace applications, and cpTi are currently the most frequently used materials for medical and dental implantation. For joint replacement, a move towards uncemented implants has changed the metal exposure from the cemented stainless steel and Co–Cr–Mo implants [Fe³⁺, Co²⁺, Cr³⁺, Ni²⁺, Cr₂O₃, CoO, Cr(OH)₃, Co(OH)₂] towards Ti oxide alloys (Ti⁵⁺, Al³⁺, TiO₂, Al₂O₃) (11). Ti alloys are superior to stainless steels and Co–Cr alloys with regard to biocompatibility and corrosion resistance, and have lower moduli of elasticity, which minimizes the risk of stress shielding of the periprosthetic bone (12). Even better shear strength and surface wear properties in certain loading conditions are desired, and other Ti alloys are thus under development, such as Ti–6Al–7Nb, Ti–15Sn–4Nb–2Ta–0.2Pd, and Ti–5Al–3Mo–4Zr (7, 13, 14), as well as surface-modified Ti alloys (15). Finally, it is important to recognize that so-called commercially pure Ti has been shown to contain impurities of other metals, for example nickel, which may have clinical significance (16).

Jewellery

Ti has been used in jewellery, particularly rings, body piercings, and watch cases, for decades (17, 18). Most often, Ti–Al–V is used, but cpTi is also used. In addition, Ti may be alloyed with gold to produce harder high-carat gold. In a study by Hamann et al. (18), a total of 956 metallic jewellery components from Europe, the United States and Japan were examined with X-ray fluorescence spectroscopy. Approximately 4% contained Ti, with a median Ti concentration of 23%.

Cosmetics and personal care

In powder form, Ti is one of the whitest substances available, making it a critical ingredient in a wide range of industrial and consumer products, including cosmetics and sunscreens. As many applications of TiO₂ benefit from smaller primary particle sizes, the use of nanosized TiO₂ is expected to increase exponentially (9). Notably,

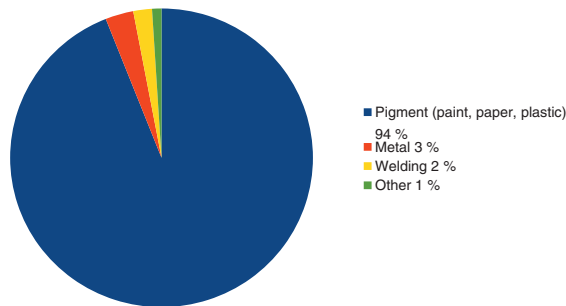


Fig. 1. Global consumption of titanium. From <http://www.titaniumcorporation.com/s/Titanium.asp> (retrieved 03 August 2014).

TiO₂ is now the third most often used material in consumer products (19). The Woodrow Wilson database has shown that ~5% of 1317 products contained TiO₂ NPs in 2011 (20). The contents of TiO₂ in personal care products have been examined in a sample pool of eight toothpastes and 24 additional personal care products (21). The TiO₂ contents in all toothpastes were in the range of 0.7–5.6 µg/mg, or from <0.1% to nearly 0.5% by weight of the product. Increased public awareness of the harmful effects of excessive ultraviolet light exposure has greatly increased the use of sunscreen products in developed countries. The use of TiO₂ NPs in sunscreens has created a vehicle that is more transparent, less viscous and blends into the skin more easily than the traditional ZnO-containing thick and cosmetically unappealing ones (22). Sunscreens generally include approximately 5–10% of TiO₂ NPs, and, routinely, 100 µl of sunscreen (equivalent to 5–10 mg Ti NPs) is used on the face each time (23).

Food

TiO₂ is a common food additive (24). A screening of 89 foods revealed that TiO₂ concentrations spanned from 0.00077 to 210 µg Ti/mg product (21). Generally, foods with high concentrations of TiO₂ include sweets or candies, chocolate, chewing gum, and foods with white icing and sugar toppings. Several dairy products with white colour, including milk, cheese, and yogurt, have TiO₂ concentrations comparable to those in non-dairy substitutes such as soy-based and rice-based drinks, in which concentrations are in the range of 0.10–0.26 µg Ti/ml (20). A human exposure analysis of TiO₂ from foods identified children as having the highest exposures, partly because the TiO₂ content of sweets is higher than that of other food products, and that a typical exposure for a US adult may be on the order of 1 mg Ti/kg body weight/day (21).

Skin Penetration of Ti

The widespread topical use of TiO₂ microparticles and NPs has led to speculation about whether these, especially in cosmetic products, have the potential to penetrate through the stratum corneum, reach the viable skin layers, and ultimately result in sensitization or other side-effects. As NP mobility and toxicity may be a function of aggregate size and increase as size decreases (25), photo-induced disaggregation could have effects on the dermal penetration of NPs (26). However, this was refuted by Miquel-Jeanjean et al. (27), who found that TiO₂ NPs in sunscreens remained in the uppermost layers of the stratum corneum in pig ears *in vitro*, regardless

of whether the skin was intact, injured, or exposed to simulated solar radiation. Several studies on the penetration of TiO₂, both coated and non-coated, through human skin have been conducted both *in vivo* and *in vitro* (Table 1). None of these showed penetration through the epidermis and into viable skin layers. A thorough review of the factors that are critical for assessing the dermal absorption of metal oxide NPs from sunscreens applied to humans was published in 2015 (51). Also, Larese Filon et al. (52) determined the hazard associated with TiO₂ NPs to be very low with regard to penetration and permeation.

Mucosal Penetration of Ti

The oral cavity is exposed to TiO₂ from toothpastes and foods, and to Ti from dental alloys and airborne particles. Structurally, the skin and the oral mucosa are similar, as they are both stratified squamous keratinized epithelia. However, there are important differences that affect their permeability kinetics. Within the granular and keratinized layers of the oral mucosa, lipids, such as (acyl)ceramides from membrane-coating granules, are deposited intercellularly to form an effective barrier (53). The oral keratinized epithelium (gingiva and palate) appears to have 25–50% less (acyl)ceramides than the epidermis, which might explain the greater permeability of this epithelium than of the epidermis (54). Unsurprisingly, the non-keratinized part of the oral mucosa is even more permeable, with the floor of the mouth being the most permeable (53), as this epithelium also lacks (acyl)ceramides. Thus, it has been stated that its permeability is up to 4000 times higher than that of the skin (55). However, the non-keratinized epithelium contains membrane-coating granules, which form an amorphous intercellular barrier material that limits the penetration of larger molecules, such as toxins, enzymes, and pharmaceuticals (53). *Ex vivo* penetration experiments with fresh porcine buccal mucosa in static Franz diffusion cells showed that different TiO₂ NPs of different size permeated the mucous layer and penetrated into the oral epithelium (56).

The oral cavity also contains a unique so-called junctional epithelium, which forms the transition from the bottom of the gingival sulcus to the cemento-enamel junction of the tooth (57). It is a stratified squamous non-keratinized epithelium of two strata only, a basal layer and a suprabasal layer. It lacks membrane-coating granules and is therefore highly permeable. As the cells are interconnected by a few desmosomes only, the intercellular spaces are relatively wide, allowing for fluid secretion and transmigration of leukocytes, the basis for the

Table 1. Titanium skin penetration

Author	Country	Year	Substance (coating)	Particle size	Method	Result
Human skin						
Tan et al. (28)	Australia	1996	TiO ₂ (NR)	NR	Human skin, <i>in vitro</i>	No penetration into skin
Dussert et al. (29)	France	1997	TiO ₂ (NR)	50–100 nm	Human skin, <i>in vitro</i>	Penetration into upper layers of SC
Lademann et al. (30)	Germany	1999	TiO ₂ (stearic acid)	150–170 nm	Human skin biopsy	Penetration into upper layers of SC; <1% of applied particles in a follicle
European Union (31)	EU	2000	TiO ₂ (anatase and rutile)	14 nm to 200 µm	Human skin, tape stripping or biopsy, pig skin (with/without UVB radiation), <i>in vitro</i>	Penetration into SC
Bennat and Müller-Goymann (32)	Germany	2000	TiO ₂ (NR)	NR	Human skin, <i>in vitro</i> + <i>in vivo</i>	Penetration into SC. Deeper penetration in oily dispersion than in aqueous dispersion
Pflucker et al. (33)	Germany	2001	TiO ₂ (SiO ₂ , Al ₂ O ₃ , SiO ₂ /Al ₂ O ₃)	10–100 nm	Human skin, <i>in vitro</i>	Penetration into upper layers of SC
Schulz et al. (34)	Germany	2002	TiO ₂ (SiO ₂ , SiO ₂ /Al ₂ O ₃)	10–100 nm	Human skin biopsy	Particles in outermost surface of SC
Popov et al. (35)	Russia	2005	TiO ₂ in sunscreen (NR)	25–200 nm	Human skin, <i>in vivo</i>	Most TiO ₂ in upper layers of SC
Mavon et al. (36)	France	2007	TiO ₂ (SiO ₂)	20 nm	Human skin, <i>in vitro</i>	Penetration into upper layers of SC
Lekki et al. (37)	Poland	2007	TiO ₂ in sunscreen (NR)	20 nm	Porcine and human skin, rubbed, <i>in vivo</i>	No penetration into viable skin layers. Penetration into follicles
Kiss et al. (38)	Hungary	2008	TiO ₂ in sunscreen (NR)	NR	Human skin on mice, <i>in vivo</i>	Penetration into SC only
Filipe et al. (39)	France	2009	TiO ₂ in sunscreen + TiO ₂ (NR)	20 nm (in sunscreen NR)	Human skin biopsy	Penetration into SC only
Crosera et al. (40)	Italy	2015	TiO ₂ in synthetic sweat (NR)	NR (nanosize)	Human skin, intact or damaged, <i>in vitro</i>	Ti detectable only in the epidermis
Animal skin						
Lansdown and Taylor (41)	UK	1997	TiO ₂ (NR)	<2–20 µm	Rabbit, <i>in vivo</i>	Penetration into SC and outer follicle
Pflucker et al. (42)	Germany	1999	TiO ₂ (trimethyloctylsilane)	20 nm	Porcine skin, <i>in vitro</i>	Penetration into upper layers of SC
Menzel et al. (43)	Germany	2004	TiO ₂ (NR)	45–150 nm	Pig skin, <i>in vitro</i>	Penetration into SC and minimal penetration into stratum granulosum
Gamer et al. (44)	Germany	2006	TiO ₂ (dimethicone)	30–60 nm	Pig skin, <i>in vitro</i>	Penetration into upper layers of SC. Almost the total amount of applied Ti could be removed from the skin surface by washing
Wu et al. (25)	China	2009	TiO ₂ (none)	4–90 nm	Pig and mouse, <i>in vivo</i> + pig, <i>in vitro</i>	<i>In vitro</i> : only small amounts detected in SC. <i>In vivo</i> (pigs): penetration into deeper layers of epidermis (only 4-nm particles). <i>In vivo</i> (mice): particles (up to 60 nm) detected in skin and various organs
Senzui et al. (45)	Japan	2010	TiO ₂ in silicone fluid, different types, coated and non-coated	35–250 nm	Porcine skin, intact, stripped, or hair removed, <i>in vitro</i>	No penetration into viable skin layers. Some penetration into follicles
Sadrieh et al. (46)	USA	2010	TiO ₂ in sunscreen (non-coated and dimethicone/methicone copolymer)	20–500 nm	Minipig skin, <i>in vivo</i>	No significant dermal penetration; some uncertainty regarding detection method
Monteiro-Riviere et al. (47)	USA	2011	TiO ₂ in water/oil (silica)	90–460 nm	Porcine skin, UVB-damaged, <i>in vivo</i> and <i>in vitro</i>	No transdermal absorption detected. Deeper penetration into UVB-damaged skin
Kimura et al. (48)	Japan	2012	TiO ₂ in sunscreen (NR)	NR	Porcine ear skin, <i>in vitro</i>	Distribution into in grooves and follicles. No penetration into viable epidermis
Bennett et al. (26)	USA	2012	TiO ₂ (none)	27 ± 4 nm	Porcine skin, <i>in vitro</i> (± sunlight or 75-W xenon arc lamp)	Light-exposed: Penetration into epidermis and dermis. Dark conditions: penetration into upper layers of SC
Miquel-Jeanjean et al. (27)	France	2012	TiO ₂ in sunscreen, different forms (some alumina-coated)	≥20 nm	Porcine skin, <i>in vitro</i> (intact, damaged, irradiated, and damaged/irradiated)	Penetration into upper layers of SC only
Adachi et al. (49)	Japan	2013	TiO ₂ in water/oil (none)	17 nm	Hairless rat skin, <i>in vivo</i>	Only penetration into SC and follicles
Peira et al. (50)	Italy	2014	TiO ₂ in water/oil (non-coated, and coated with trimethoxycaprylsilane and amorphous silica)	~15–52 nm	Porcine skin, <i>in vitro</i>	Penetration into SC

NR, not reported; SC, stratum corneum; UVB, ultraviolet B.

crevicular fluid, which comprise the first line of peripheral host defence against bacteria. In a situation of inflammation, the epithelial attachment may be lost or the junctional epithelium may even become disrupted, because of either increased fluid flow or bacterial products and leukocytes passing through (57). Especially when the tissue is inflamed, the junctional epithelium has been shown to be permeable to a variety of materials, ranging from carbon particles to proteins (58, 59).

In vivo, all oral epithelia, except for the junctional epithelium, are covered by a layer of saliva. Saliva has an important cleansing function, but it also contains mucins that bind covalently to the epithelium surface. The latter serves to concentrate secretory IgA and lysozymes, and this in turn limits the attachment of microorganisms. Ti NPs rapidly interact with the mucous layer, penetrate the underlying tissue within minutes, and impact on the physiological homeostasis of buccal/sublingual cells in the oral cavity (56, 60, 61). NP surface hydrophilicity/hydrophobicity impacts on the distribution within the mucosa. Hydrophilic rutile 20-nm TiO₂ NPs were freely distributed in the cytoplasm, whereas their hydrophobic counterparts were engulfed in vesicular structures. Although cell viability/membrane integrity was not affected negatively, hydrophilic TiO₂ NPs showed a higher potential to decrease the physiological mitochondrial membrane potential than hydrophobic TiO₂ NPs, resulting in a pronounced generation of reactive oxygen species (62).

Release of Ti Ions from Metal Alloys

A spontaneously formed stable oxide film (5–10 nm) composed primarily of TiO₂, created upon exposure to air and biological fluids, provides pure Ti and Ti-based alloys with outstanding resistance to corrosion (63). Despite this, release and *in vivo* studies have proven that the oxide layer can be compromised, and ions and particles are indeed released from cpTi and Ti alloys into biological fluids and tissue (Table 2). The protective oxide film is composed primarily of TiO₂, which is highly stable. The oxide film could result from bulk Ti alone, and the alloying elements (Mo, Nb, V, Cr, etc.) would not be present in the passive film to any significant extent. However, from a microscopic point of view, it has been shown that the passive film is continuously dissolved and reconstructed in aqueous solutions, and dissolution of alloying elements is therefore possible. Moreover, incorporation of different elements from the solution into the film, for example repassivation of Ti in biological liquid, can lead to the adsorption of calcium and phosphate ions into the film. In turn, calcium phosphate and calcium titanium phosphate are formed at the outermost

surface (95). Incorporation of other metals in the TiO₂ passive layer might weaken the protective properties, and this might explain the poorer stability of Ti alloys than of TiO₂ materials, and explain the differences in stability between different Ti alloys despite formation of the oxide layer. The stability of the protective oxide layer also depends on the electrode potential and the pH of the biological fluid, which in turn can be affected by cells, bacteria, inflammation, lipopolysaccharides (found in the outer membrane of Gram-negative bacteria), and the availability of oxygen. Besides the general corrosion, metal ions could also be released if the thin oxide layer were disrupted mechanically, and during repassivation Ti ions could be released from the bulk material. Finally, corrosive milieu proteins (e.g. albumin, α -globulin, transferrin, and fibrinogen) and amino acids have a strong affinity for metal ions, and, because these elements are capable of forming metallo-organic complexes, they will increase the corrosion rate of Ti alloys significantly (6, 66, 68, 72, 74, 76–78, 81–83, 90, 96–105). The most obvious evidence of Ti release or corrosion is a dark, probably harmless, tissue discolouration that has been reported in both stable and failed implants, owing to oxide produced after surface wear (106–108) (Fig. 2). Histologically, foreign body giant cells are rare in this tissue, and retrieved particles are almost identical to the bulk material, indicating that wear is a likely mechanism for generating the debris (109). Hence, the metal ions are corroded off the metallic implant, but may also come from corrosion of metallic wear debris. The surface area (surface area/mass) of metallic particles may result in high corrosion rates, owing to the large area exposed to the corrosive environment (110).

A study by Cadosch et al. (111) showed that human osteoclast precursors are able to grow and differentiate to form mature osteoclasts on Ti and aluminium. Furthermore, it established that the mature cells are able to directly corrode the metal surface and take up corresponding metal ions, which may subsequently be released and thereby induce the formation of osteolytic lesions in the periprosthetic bone, contributing to the loosening of the implant. Jacobs et al. (86) found that serum concentrations of Ti were elevated approximately twofold in patients who had a loose implant as compared with patients with a well-functioning implant and controls with no implant, and several studies of both humans and animals with inserted implants have shown an increased presence of Ti ions in surrounding tissue, serum, urine, peri-implant tissues, regional lymph nodes, spleen, adjacent muscle, and lungs, particularly in combination with loosened implants (79–81, 84, 87–89, 94). However, a similar number of studies found no significant

Table 2. Titanium corrosion and release; selected studies

Author	Country	Year	Materials and method	Result
Strietzel et al. (64)	Germany	1998	Pure Ti immersed in different solutions	Ti revealed ion release [0.01–0.1 $\mu\text{g}/(\text{cm}^2 \times \text{day})$] from the majority of gold alloys. There was little influence of grinding and casting systems in comparison with organic acids or pH value. The Ti ion release increased greatly up to 500 $\mu\text{g}/(\text{cm}^2 \times \text{day})$ in the presence of fluoride. Low pH values accelerated this effect even more
Gil et al. (65)	Spain	1999	Ti alloy in artificial saliva	Low value of Ti ion release and good corrosion resistance
Koike and Fujii (66)	Japan	2001	Pure Ti immersed in lactic and formic acids at pH 1.0–8.5	The amount of dissolved Ti tended to decrease with higher pH. The Ti samples immersed in lactic acid all decreased in weight, but this was not affected by pH. In formic acid, the weight decreased at pH 1.0 and increased at pH 2.5–5.5
Nakagawa et al. (67)	Japan	2001	Pure Ti and different alloys immersed in 0.05–2.0% NaF at different pH	Pure Ti, Ti–6Al–4V and Ti–6Al–7Nb alloys were easily corroded even in a low fluoride concentration in an acidic environment. The corrosion resistance of Ti–0.2Pd alloy was greater than those of pure Ti, Ti–6Al–4V and Ti–6Al–7Nb alloys in the wide range of pH and fluoride concentrations
Koike and Fujii (68)	Japan	2001	Cast pure Ti immersed in different solutions	The weight changed and varied with different immersion solutions. The thickness of surface TiO_2 also varied
Huang et al. (69)	Taiwan	2003	Different Ni–Ti orthodontic wires in artificial saliva with various pH values	Detectable Ti release at pH 2.5. Ti ions were mostly not detectable in pH ≥ 3.75 solution after either a 1-day or a 28-day immersion period
Gioka et al. (70)	Greece	2004	Two brands of Ti orthodontic brackets immersed in 0.9% saline	Released Ti levels were below the threshold level (1 ng/ml) of analysis
Wennerberg et al. (71)	Sweden	2004	Ti of different roughness immersed in solutions plus Ti implanted in rabbit tibiae	Ti ion release, but no significant difference between the different surfaces. <i>In vivo</i> , Ti rapidly decreased with distance from the implant surface after 12 weeks and 1 year
Okazaki and Gotoh (72)	Japan	2005	Pure Ti and different alloys immersed in different solutions	The quantities of Ti released into alpha-medium, PBS(–), calf serum, 0.9% NaCl and artificial saliva were much lower than those released into 1.2% L-cysteine, 1% lactic acid and 0.01% HCl. The quantity of Ti released from the Ti–15Zr–4Nb–4Ta alloy was smaller than those released from the Ti–6Al–4V and Ti–6Al–7Nb alloys in all of the solutions. The quantity of Ti released increased with decreasing pH
Molina et al. (73)	Spain	2008	Pure Ti immersed, brushed without paste, or brushed with one of four toothpastes of different relative dentine abrasiveness	Ti released from tooth brushing, but did not reach 40 ppb
Hirayama et al. (74)	Japan	2008	Pure Ti immersed in different solutions	Significantly larger amounts of dissolved Ti ions in mixed organic acid solutions than in lactic acid solution. There was also a significant difference in the weight loss, but the significance level was different from that of the difference in the quantity of Ti ion elution

Table 2. Continued

Author	Country	Year	Materials and method	Result
Kretzer et al. (75)	Germany	2009	Ti THA in an <i>in vivo</i> simulated experimental set-up	No mechanical failure or excessive corrosion could be identified for the implants tested. The measured amounts of Ti released were extremely low as compared with <i>in vivo</i> and <i>in vitro</i> studies, and were not in a critical range
Joseph et al. (76)	Nigeria	2009	Pure Ti and Ti–Al6–Nb7 immersed in different solutions and at different pH	Ti release from both pure Ti and alloy. Ti ions from pure Ti were released from day 1 after immersion, but were not released from the alloy until after 12 weeks. The results showed that variations in pH and chloride ion level of the test medium has a significant effect on the amounts of Ti ions released. The average rates of Ti release over a 30-day period were 1.52, 1.69 and 1.99 pg/cm ² /s
Zhang et al. (77)	UK	2010	Ti treated by DC plasma electrolytic oxidation in three different electrolytes immersed in simulated body fluid	Ti ion release was highest in artificial saliva of pH 2.3 and lowest in 0.9% saline solution of pH 7.3
Mutlu-Sagesen et al. (78)	Turkey	2011	Ti-based alloy immersed in artificial saliva and 0.9% saline solution at different pH	In killed baboons with implants, increased Ti levels were noted in the lungs, spleen, adjacent muscle, and regional lymph nodes. In living baboons with implants, a sixfold increase in the urine Ti level was noted
Woodman et al. (79)	USA	1984	Forty-five baboons with Ti implants. Thirty-eight killed, and 7 kept alive for blood and urine samples. Thirteen controls	
Schliephake et al. (80)	Germany	1993	Ti screw taps and fixtures before and after experimental use in minipigs	Ti particles were abraded and deposited on the bone surface during implantation. Five months later, particles were no longer observed. Determination of the Ti concentration of the inner organs showed that the lungs contained the highest amount of Ti
Weingart et al. (81)	Germany	1994	Insertion of endosseous Ti screw implants in 19 beagle dogs. Five controls	Very fine foreign body particles and a significantly higher concentration of Ti in the lymph nodes in the group with implants
Martinez et al. (82)	USA	1995	Ti–6Al–4V posterior cervical spine plates in canines	The highest Ti levels (>100 ppm dry weight) and the most severe surface damage were associated with screw plate interfaces
Bianco et al. (83)	USA	1996	Ti fibre felts implanted into the tibias of 36 rabbits for periods up to 1 year. Seven controls. Seven rabbits had sham surgery	After 1, 4 and 12 months, in the absence of wear, Ti accumulated locally in bone and muscle. The authors concluded that metal species released from Ti implants in the absence of wear have limited solubility
Friskien et al. (84)	Tasmania	2002	Ti implant in mandibles of 12 sheep. Four controls	The results associated with successful implants showed no statistically significant different levels of Ti in any organ as compared with controls. Two implants failed to integrate, and these showed higher levels of Ti in the lungs and regional lymph nodes
Lee et al. (85)	Singapore	2009	Ti mandibular implant in monkeys plus controls	No detectable Ti particles in the mucosa surrounding the endoprosthesis, or in the regional lymph nodes and distant organs. Ti was detectable in blood, but there was no significant difference between the groups

Table 2. Continued

Author	Country	Year	Materials and method	Result
Jacobs et al. (86)	USA	1991	Patients with a stable or loose Ti-containing THA	Serum concentrations of Ti were elevated approximately twofold in the patients who had a loose implant. There were no major differences in terms of urine concentrations of Ti
Torgersen et al. (87)	Norway	1995	Ti miniplates removed after healing of jaw fracture	Ti particles were found in both soft tissue and bone next to implants
Jacobs et al. (88)	USA	1998	Fifty-five patients with Ti-containing THAs. Twenty controls	Thirty-six months postoperatively, patients with a well-functioning Ti-containing implant had as much as a threefold increase in the concentration of Ti in serum
Jacobs et al. (89)	USA	1999	Patients with stable or loose Ti-containing TKAs of different designs plus controls without implants	Serum concentrations of Ti were ~50 times greater in patients with failed patellar components and ~10 times greater in patients with carbon fibre-reinforced polyethylene-bearing surfaces than in patients with other designs and control subjects. Analysis of 24-h urine samples showed no significant differences in Ti concentrations
Leopold et al. (90)	USA	2000	Case: Ti patellar component in TKA	Failed Ti patellar component and serum Ti elevated 98 times as compared with pre-implantation
Harzer et al. (91)	Germany	2001	Ti brackets placed on left molar bands, and stainless steel brackets placed on right bands. Fifteen patients used fluoride-containing toothpaste, and 3 used fluoride-free toothpaste	Pitting and crevices were observed in only 3 of the 165 brackets tested. There were no significant differences among the brackets, wires and toothpastes used
Meninguad et al. (92)	France	2001	Removal of Ti miniplates after maxillofacial surgery	The average amount of total Ti in the soft tissues surrounding the plates was 1306 µg/g dry tissue. The mean of soluble Ti was 0.53 µg/g dry tissue. There was no relationship between the duration of plating and total Ti, and nor was there soluble Ti in the soft tissue surrounding the plates. Almost 100% of the Ti was found to be released during osteosynthesis
Savarino et al. (93)	Italy	2006	Stable Ti-containing THA, ceramic-on-ceramic or metal-on-metal, plus controls	The serum concentration of Ti did not show any statistical difference between the groups
Savarino et al. (94)	Italy	2010	Patients with stable or loose first Ti-containing TKA plus controls	No significant difference in serum Ti concentrations between the groups

PBS, phosphate-buffered saline; THA, total hip alloplasty; TKA, total knee alloplasty.

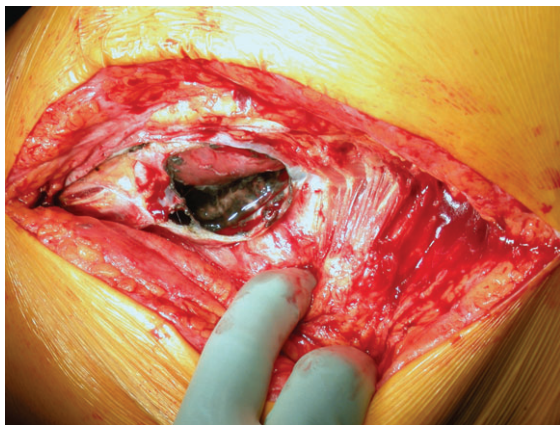


Fig. 2. Metallosis following a total hip arthroplasty in the left hip.

difference between study subjects/animals and controls, or significant release *in vitro* (65, 69–71, 83, 85, 92, 93, 112, 113).

With regard to dental implants, several factors in the oral environment do indeed lead to increased metal release and possibly Ti release as compared with other forms of implant. These factors include the constant presence of some level of infection (gingivitis or periodontitis) and lipopolysaccharides (constant presence of bacterial load), the use of fluoride-containing toothpastes and tooth brushing, the use of mouthwashes, higher glucose levels, and the corrosive environment itself, including the presence of saliva containing corrosive compounds such as hydrogen, chloride ions, sulfide compounds, dissolved oxygen, and free radicals, and with changing pH. As previously stated, corrosion is promoted in acidic environments, but also by the presence of lipopolysaccharides (66, 105, 114). In healthy adults, the salivary pH level ranges from 6.3 to 7 (105). Acidic foods, such as soft drinks and fruits, and the presence of infection will reduce the pH of saliva to approximately 2–3 (115). Toothpaste not only leads to abrasion of metal surfaces, but also contains fluoride, which has a noxious effect on Ti in high concentrations (116). Although there are conflicting data (73), immersion studies of cpTi and alloys in fluoride and bleaching agents have shown evidence of corrosion (64, 67, 102, 117).

In this regard, it is also important to consider that the mucosa and the skin behave differently, owing to the influence of specific immune systems for each, such as skin and mucosa-associated lymphoid tissue. The number of Langerhans cells is much lower in the oral mucosa, for example, and it must thus be exposed to allergen concentrations 5–12 times greater than the skin in order to cause tissue reactions (6). Moreover, different

subtypes of immune cells reside in the oral mucosa, and these primarily react in a tolerogenic manner upon contact with antigens such as lipopolysaccharides (118). This explains why patients who are allergic to nickel may still tolerate nickel-containing orthodontic appliances, and why oral disease is seen relatively infrequently despite the continuous attack by pathogenic bacteria and other exogenous antigens. In addition, oral exposure to metals such as nickel and cobalt, for example by wearing orthodontic braces, before cutaneous exposure leads to immunological tolerance (119, 120). Similar reservations apply to allergic reactions following implantation.

Immunology

In general, there are four groups of allergens. Haptens are classified as low molecular weight chemicals with an intrinsic potential to covalently modify major histocompatibility complex-binding self-peptides. Many of them are strong inducers of contact hypersensitivity. Prohaptens are classified as molecules that are only transformed into reactive metabolites able to modify proteins upon cellular metabolism. Prehaptens are non-sensitizing or low-sensitizing chemicals that are transformed into haptens outside the skin/body by chemical transformation (air oxidation; photo-activation) without the requirement for specific enzymatic systems (121). The fourth group comprises transition metals, and, in contrast to classic haptens, metal ions do not form stable covalent protein modifications. Rather, they produce geometrically highly defined coordination complexes, which, by definition, are reversible and allow the exchange of the allergenic metal ions between different binding sites. Thus, it has been difficult to define allergenic metal epitopes. Like nickel, the most common contact sensitizer in the industrialized world, Ti is a transition metal. Data on the molecular basis of T cell activation by nickel imply that metal ions, unlike classic haptens, are capable of activating specific T cells by a variety of molecular mechanisms (122). The exact mechanism by which nickel induces allergic reactions is not yet fully understood, and, even more so, the same applies for Ti. Hence, the reason why, for example, Ti and zinc are so much less allergenic than nickel and other transition metals, despite the actual release of ions into biological fluids, cannot yet be outlined. Park et al. (17) analysed the sensitization potential of TiO₂ with the local lymph node assay, and found TiO₂ NPs not to be dermal sensitizers. No studies on allergy to Ti alloys or Ti NPs have, to our knowledge, been conducted with the guinea-pig maximization test.

Immune reactions caused by Ti alloys

Ti has been reported to activate macrophages, either directly or subsequent to phagocytosis. *In vitro* phagocytic uptake of Ti by macrophages was clearly shown in a study by Müller et al. (123, 124), and such activated macrophages may secrete both pro-inflammatory and anti-inflammatory cytokines, an imbalance that has been implicated in various disease processes and allergic reactions. Thus, Nakashima et al. (125) found that Ti alloy particles increased the expression of certain chemokines in macrophages *in vitro* in a dose-dependent and time-dependent manner. Also mRNA signal levels of these chemokines were observed after exposure, and monocyte migration was stimulated by culture medium collected from macrophages exposed to Ti alloy particles. In a study by Lalor et al. (107), tissues from 5 patients who underwent revision surgery for failed total hip replacements were found to contain large quantities of particulate Ti. Monoclonal antibody labelling showed abundant macrophages and T lymphocytes in the absence of B lymphocytes, suggesting sensitization to Ti. In contrast, Flatebø et al. (126) inserted Ti dental implants into 13 patients without previous implants. Six-month, but not baseline, biopsies contained dense particles that were most likely metals, but tissue sensitivity reactions to Ti implants were not found. Wang et al. (100) investigated immune response and the release of immunoregulatory cytokines after peritoneal injection of Ti into mice. The results indicated that metal-induced immunosuppression might be an important factor in the development of implant-associated infection in patients with a prosthesis.

Immune reactions caused by Ti NPs

Larsen et al. (127) found that TiO₂ NPs promoted a Th2-dominant immune response with high levels of specific IgE and IgG₁ in serum, and the influx of eosinophils, neutrophils and lymphocytes into bronchoalveolar lavage fluid. Mice were immunized via intraperitoneal injections of ovalbumin (OVA), OVA + TiO₂, or OVA + AlOH₃, and challenged with aerosols of OVA. Yazdi et al. (128) showed that TiO₂ NPs activate the NLR pyrin domain-containing 3 (Nlrp3) inflammasome, which is involved in inflammation and other immune responses, leading to interleukin (IL)-1 β release, and in addition induce the regulated release of IL-1 α . Unlike what is seen with other Nlrp3 agonists, TiO₂ NP-dependent Nlrp3 activity did not require phagocytosis and induced IL-1 α / β secretion in non-phagocytic keratinocytes. In an experimental model of NC/Nga mice assumed to show skin barrier dysfunction, Yanagisawa et al. (23) showed that intradermal

exposure to TiO₂ NPs could aggravate atopic dermatitis such as skin lesions. The effects were paralleled by enhancement of IL-4 expression in the ear, the levels of total IgE and histamine in serum, and a reduction in interferon- γ expression. Additionally, TiO₂ NPs alone significantly increased histamine levels in serum and IL-13 expression in the ear. The authors suggested that TiO₂ NPs, under conditions of skin barrier dysfunction, may aggravate atopic dermatitis, and may play a role in the initiation and progression of skin diseases following the barrier dysfunction by histamine release – even in the absence of an allergen. In support of this, an immunomodulatory ability of Ti NPs was shown in an animal study, when administration of TiO₂ NPs increased the dermal sensitization potency of 2,4-dinitrochlorobenzene, a well-known sensitizer, by augmenting a Th2 response (19). However, in an experimental study from 2011 using cultured keratinocytes, a human skin-equivalent model, and an animal model, Park et al. (17) concluded that TiO₂ NPs induced no phototoxicity, acute cutaneous irritation, or skin sensitization. Local lymph node assay results from the same study indicated that TiO₂ NPs were not in themselves dermal sensitizers. In conclusion, Ti NPs can certainly exert reactions involved in allergy and other inflammatory responses, but the significance of these events and the issue about exposure, especially dermal exposure, remains to be further investigated, particularly in humans.

Regarding non-allergic adverse effects, exposure of cell cultures to TiO₂ NPs has been shown to result in decreased cell area, proliferation, mobility, and ability to contract collagen (4, 129). Exposure of gingival cells to Ti resulted in increased expression of toll-like receptor 4 and intercellular adhesion molecule 1 (130). Indeed, these data indicate that Ti ions may be involved in cytotoxicity and inflammation, without sensitization, and certainly Ti alloys and debris might cause toxic reactions on tissue and in cell cultures. However, the data are conflicting, and the extent and clinical significance in the long term are unclear (79, 131, 132).

Diagnosing Ti Allergy

A diagnosis of Ti allergy has typically been based on medical history, clinical findings, and the results from patch testing. However, low epidermal penetration of commercially available Ti salts makes patch testing unreliable (133, 134). Examples of some commercially available materials for patch testing are calcium titanate (10% pet.), Ti(III) nitride (5% pet.), TiO₂ (10% pet.), Ti(III) oxalate decahydrate (5% pet.), and Ti (10% pet.) (135). The presence of impurities (Al, Be, Ni, Cd, Co, Cr, Cu, Fe,

Hf, Mn, Mo, Pd, and V) in Ti materials, resulting from the production process, is important to consider when patch test results are interpreted. Indeed, the small amounts are insignificant from a metallurgical perspective, but may be sufficient to trigger allergic reactions in patients sensitized to the corresponding allergen (136). Hence, reported cases of suspected Ti allergy have, after further investigations, been found or suspected to be caused by other allergens or causes see Clinical Reactions and Case Reports.

In vitro blood tests are used by a few physicians to detect metal allergy. Common tests include the lymphocyte transformation test (137–145), the lymphocyte migration inhibition test (146–149), and the commercially available memory lymphocyte immunostimulation assay (MELISA®) (150). Additionally, flow cytometry has been suggested as a method for assessing allergy to metal implants (151). None of these tests has yet been generally accepted as an optimal method for detecting allergy, owing to insufficient validation, costs, lack of access to centres performing such tests, variable test results, and possible interlaboratory variations (152). Although MELISA® is a widely published *in vitro* test, and a number of articles have suggested its clinical utility, it is not approved as a routine method for testing Ti allergy, and is still under evaluation. An *in vitro* study (153) reported no significant difference between MELISA® and conventional lymphocyte transformation tests in terms of sensitivity and specificity. It has been suggested that these tests are not useful in the diagnosis of allergy to metals, because of the low specificity (between 17% and 79%). However, testing was not performed with Ti, and no direct comparison in a single patient was made. The sensitivity, specificity and reproducibility of MELISA® against up to 20 different metals, including Ti, were analysed in a recent study (150) of 250 patients with a suspected type IV allergy to a metal. The authors concluded that MELISA® was reliable for detecting metal allergy. However, no testing was performed on healthy controls in general, and there is only sparse information on whether the suspicion of metal allergy in the different patients was based on the patients' histories and clinical findings alone or on other test results. Müller and Valentine-Thon (124) reported on 56 patients with various clinical symptoms after receiving Ti-based implants. None showed a positive patch test result. With MELISA® using TiO₂, 21 (37.5%) were positive, with an average stimulation index (SI) of 6.3, 16 (28.6%) were ambiguous, with an average SI of 2.4, and 19 (33.9%) were negative (SI < 2). Fifty-four patients had the implant removed, and all showed clinical improvement. It should be noted that, in this study, 57.9% of the patients reacted to other metals, including Ni. The results could represent an

overestimation of the actual prevalence, owing to the suspected low specificity of MELISA®. No controls were tested. Hallab et al. (134) suggested that a multi-assay system, with each assay measuring a different aspect of lymphocyte/monocyte-mediated reactivity, may help to improve the detection of metal-induced allergy associated with metallic implants over that of a single assay system. In conclusion, the clinical history remains very important, particularly when Ti allergy to an implant is suspected. In 2011 (154), 10 points were suggested that could strengthen the suspicion of clinically relevant metal allergy to an orthopaedic implant: (i) chronic dermatitis beginning weeks to months after metallic implantation; (ii) an eruption overlying the metal implant; (iii) a morphology consistent with dermatitis (erythema, induration, papules, and vesicles); (iv) in rare instances, a systemic allergic dermatitis reaction (characterized by universal dermatitis reactions, typically localized in body flexures); (v) histology consistent with allergic contact dermatitis; (vi) a positive patch test reaction to a metal used in the implant (often strong reactions); (vii) serial dilution patch testing giving positive reactions to low concentrations of the metal under suspicion; (viii) positive *in vitro* test results for metals, for example the lymphocyte transformation test; (ix) the dermatitis reaction being therapy-resistant; and (x) complete recovery following removal of the offending implant.

There are, to our knowledge, no epidemiological studies on the prevalence of Ti allergy in the general population, and, as no reliable diagnostic test is available, the clinical test data presented in Tables 3 and 4 are probably of low reliability.

Clinical Reactions and Case Reports

Most published data concern dental and orthopaedic implants, and the cases are generally divided into either contact allergy (type IV hypersensitivity) or systemic hypersensitivity. Some of the first cases in which allergy to Ti was suspected were described in patients with cardiac pacemakers. Since then, more case reports of suspected Ti allergy to, especially, dental and orthopaedic materials have been published. The reported symptoms caused by suspected Ti allergy are diverse and, in addition to aseptic loosening of implants, include dermatitis, stomatitis, chronic inflammation in adjacent tissue, impaired wound healing, acne-like facial inflammation, drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, chronic fatigue syndrome, muscle and joint pain, and neurological problems (Table 5) (124, 182, 183). However, a significant proportion of symptoms are never conclusively interpreted as being attributable to Ti

Table 3. Patch tests for titanium hypersensitivity

Author	Country	Year	Patients	Test substance	Readings (days)	No.	Positive (no.)	Positive (%)	Comments
Elves et al. (155)	England	1975	Patients with stable or failed joint replacements of various contents	C ₄ K ₂ O ₉ Ti 1% solution	3, 7	50	0	0	–
Waterman et al. (156)	The Netherlands	1985	Patients scheduled for metal-to-polyethylene THA implants	TiO ₂ 5% pet.	2–3	149	1	0.7	Implants of various contents
Lalor et al. (107)	England	1991	Mean 17.5 months postoperatively	TiO ₂ 5% pet.	2–3	85	0	0	–
			Patients with failed Ti-containing THA, revision surgery, and histoinmunological signs of HS	C ₂₈ H ₂₀ O ₁₂ Ti, TiC ₁₆ H ₄₈ O ₄₆ , TiO ₂ , and H ₄ O ₄ Ti, each at 1%, 2% and 5% in white soft paraffin	2, 4	5	0	0	–
			Patients with failed Ti-containing THA, revision surgery, and histoinmunological signs of HS	Metanium ointment (20% TiO ₂ , 5% H ₄ O ₄ Ti, 3% C ₂₈ H ₂₀ O ₁₂ Ti, 0.1% TiC ₁₆ H ₄₈ O ₄₆)	2, 4	4	2	50	–
			Controls (2 with MHS)	Metanium ointment (20% TiO ₂ , 5% H ₄ O ₄ Ti, 3% C ₂₈ H ₂₀ O ₁₂ Ti, 0.1% TiC ₁₆ H ₄₈ O ₄₆)	2, 4	12	0	0	–
			Patients with stable or failed THA	TiO ₂ 5% pet.	–	66	1	1.5	Implants of various contents
			Controls with no implants	TiO ₂ 5% pet.	–	41	0	0	–
Cancilleri et al. (157)	Italy	1992	Patients with stable or failed THA	Ti (NR specifically)	2, 4	15	0	0	Implants were
Haddad et al. (158)	England	1996	Patients with aseptic loose THA	Ti (NR specifically)	2, 4	25	0	0	– Ti-containing
			Patients with stable THA	Ti (NR specifically)	2, 4	5	0	0	–
			Patients with septic loose THA	Ti (NR specifically)	2, 4	25	0	0	–
			Patients scheduled for THA	Ti (NR specifically)	2, 4	184	4	2.2	–
Lhotka et al. (159)	Austria	1998	Patients with metal skin clips (0.001–0.315% Ti) after surgery	TiO ₂ 1% pet.	2, 3, 4	40	0	0	No failed implants contained Ti
Milavec-Puretić et al. (160)	Croatia	1998	Patients scheduled for revision THA	Ti powder	2, 3	40	0	0	–
Okamura et al. (161)	Japan	1999	Patients with suspected MHS	TiCl ₄ 0.2% solution	2, 3, 5–7	73	1	1.4	–
				TiCl ₄ 0.1% solution	–	73	2	2.7	–
				Ti(SO ₄) ₂ 0.2% solution	–	73	1	1.4	–
				Ti(SO ₄) ₂ 0.1% solution	–	73	2	2.7	–
				TiO ₂ 20% ointment	–	73	0	0	–
			Healthy controls	TiCl ₄ 5% solution	2, 3, 5–7	2	2	100	–
				TiCl ₄ 2% solution	–	2	2	100	–
				TiCl ₄ 1% solution	–	13	4	30.1	–
				TiCl ₄ 0.5% solution	–	13	3	23.1	–
				TiCl ₄ 0.2% solution	–	13	0	0.9	–
				TiCl ₄ 0.1% solution	–	13	0	0.9	–
				Ti(SO ₄) ₂ 5% solution	–	2	2	100	–
				Ti(SO ₄) ₂ 2% solution	–	2	2	100	–
				Ti(SO ₄) ₂ 1% solution	–	13	4	30.1	–
				Ti(SO ₄) ₂ 0.5% solution	–	13	2	15.4	–

Table 3. Continued

Author	Country	Year	Patients	Test substance	Readings (days)	No.	Positive (no.)	Positive (%)	Comments
Menezes et al. (162)	Brazil	2004	Patients scheduled for orthodontic implant	Ti(SO ₄) ₂ 0.2% solution Ti(SO ₄) ₂ 0.1% solution TiO ₂ 20% ointment TiO ₂ 1%	– – – 2.3	13 13 13 38	0 0 0 2	0 0 0 5.3	– – – –
Iijima et al. (163)	Japan	2005	Two months post-implantation Patients with cardiac stent (65 with in-stent restenosis)	TiO ₂ 1% Ti 20% pet.	2.3 2.3	38 174	0 0	0 0	– Most stents contained no Ti
Granchi et al. (152)	Italy	2006	Candidates for THA Stable Ti–Al–V or Co–Cr–Mo/Ti–Al–V hip implant Loose Ti–Al–V hip implant Loose Co–Cr–Mo/Ti–Al–V hip implant	TiO ₂ 2% pet. TiO ₂ 2% pet. TiO ₂ 2% pet. TiO ₂ 2% pet.	2.3 2.3 2.3 2.3	66 53 22 25	1 0 1 0	1.5 0 4.5 0	Some patients had previous implants – – –
Müller and Valentine-Thon (124)	Germany	2006	Loose hip implant, unknown material Patients with clinical symptoms after receiving Ti-containing implants	TiO ₂ 2% pet. TiO ₂ 0.1% ointment	2.3 NR	26 54	1 0	3.8 0	– 21 (37.5%) of 56 patients tested positive in MELISA®
Granchi et al. (192)	Italy	2008	Patients with stable TKA	TiO ₂ 2% pet.	2–3	27	0	0	One with Ti–Al–V alloy and 23 with Co–Cr–Mo/Ti–Al–V alloy
			Patients with loose TKA	TiO ₂ 2% pet.	2–3	47	1	2.1	Two with Ti–Al–V alloy and 27 with Co–Cr–Mo/Ti–Al–V alloy
Sicilia et al. (193)	Spain	2008	Controls with no implant Patients with orthodontic implants and symptoms or unexplained implant failure Patients with multiple allergies or heavily exposed to Ti implants Healthy controls	TiO ₂ 2% pet. TiO ₂ 0.1% and 5% pet. + metallic Ti 0.1% and 5% aqueous solution TiO ₂ 0.1% and 5% pet. + metallic Ti 0.1% and 5% aqueous solution TiO ₂ 0.1% and 5% pet. + metallic Ti 0.1% and 5% aqueous solution	2–3 1, 2, 3 1, 2, 3 1, 2, 3	20 16 19 35	1 8 1 0	5 50 5.3 0	– Specific patch test that gave a positive result NR – –
Reed et al. (194)	USA	2008	Patients with a history of MHS scheduled for metallic implants	–	3, 5, 6, 11–14	22	0	0	–
Thomas et al. (167)	Germany	2009	Patients with failed implants Patients with failed metal-on-metal THA undergoing revision surgery	Various Ti disks TiO ₂ 0.1% (vehicle NR)	3, 5, 6, 11–14 2.3	22 16	0 0	0 0	Content of implant NR Content of implant NR

Table 3. Continued

Author	Country	Year	Patients	Test substance	Readings (days)	No.	Positive (no.)	Positive (%)	Comments
Davis et al. (195)	USA	2011	Patients with suspected MHS	TiO ₂ 1% pet. Ti 10% pet.	3, 5, 7–10, 11–17 3, 5, 7–10, 11–17	119 94	0 0	0 0	– –
Frigerio et al. (168)	Italy	2011	Patients scheduled to have first THA/TKA	Ti alloy disc Ti 10% pet.	3, 5, 7–10, 11–17 4, (7)	904 100	8 0	0.9 0	– –
Kręćisz et al. (196)	Poland	2012	1 year post-implantation Patients scheduled for hip or knee arthroplasty	Ti 10% pet. TiO ₂ 10% pet.	4, 7 2, 4	72 60	0 0	0 0	Approximately 80% with a Ti-containing implant –
Vermes et al. (169)	Hungary	2013	Twenty-four months postoperatively Controls with no implant Patients with no implant and with a history of MHS	TiO ₂ 10% pet. Ti(C ₂ O ₄) ₂ 0.5%, 1%, and 5% Ti(C ₂ O ₄) ₂ 0.5%, 1%, and 5%	2, 4 2, 3, 4, 7 2, 3, 4, 7	48 7 7	0 0 0	0 0 0	Content of implants NR – –
			Patients with no history of MHS pre-implantation Patients with no history of MHS post-implantation of Ti-containing implant	Ti(C ₂ O ₄) ₂ 0.5%, 1%, and 5% Ti(C ₂ O ₄) ₂ 0.5%, 1% and 5% pet.	2, 3, 4, 7 2, 3, 4, 7	40 40	0 0	0 0	– Patch testing at 3, 6, 12, 24 and 36 months
Bernard S et al. (136)	Belgium	2013	Healthy controls and patients with AD	TiO ₂ 10% pet.	2, 4	30	0	0	–

AD, atopic dermatitis; HS, hypersensitivity; MHS, metal hypersensitivity; NR, not reported; THA, total hip alloplasty; TKA, total knee alloplasty.

Table 4. *In vitro* tests for titanium hypersensitivity

Author	Country	Year	Patients	Test	No.	Positive (no.)	Positive (%)	Comment
Merritt and Rodrigo (147)	USA	1996	Patients scheduled for TJR	LMI	22	0	0	–
Woolley et al. (164)	USA	1996	3–12 months after implantation Patients with OA, RA or AN and either candidates for TJR or patients with aseptic loose TJR	– Cell proliferation assay	22 66	5 –	23 –	– Low proliferation indexes in all groups and no significant differences. Contents of implants NR, but Ti content reported to be rare
Stęjskal et al. (165)	Sweden	1999	Patients suspected of having MHS (various symptoms) to dental implants	MELISA®	3162	–	~5.6	–
Hallab et al. (134)	USA	2000	Healthy controls	MELISA®	116	–	~2	26% had positive patch test reactions, mostly to nickel and thimerosal
Valentine-Thon and Schwara (150)	Germany	2003	Patients with Ti implants and MHS	LTT, MIF (ELISA), LMI	3	0	0	–
Hallab et al. (166)	USA	2004	Healthy controls	LTT, MIF (ELISA), LMI	3	0	0	–
			Patients with OA	MELISA®	250	–	42	Specific exposure to Ti NR
			Patients with stable metal-on -polyethylene THA (Ti-based)	LTT	12	0	0	–
			Patients with stable metal-on-metal THA (Co–Cr)	LTT	6	0	0	–
			Patients with various symptoms after receiving a Ti-based implant	LTT	7	0	0	–
Müller and Valentine-Thon (124)	Germany	2006	Patients with stable metal-on-metal THA (Co–Cr)	LTT	9	0	0	–
			Patients with various symptoms after receiving a Ti-based implant	MELISA®	56	21	37.5	Fifty-four of the patients were negative in patch testing
			Six months after removal of Ti-based implant	MELISA®	15	0	0	All patients reported clinical improvement after removal of implant
Thomas et al. (167)	Germany	2009	Patients with failed metal-on-metal THA undergoing revision surgery	LTT	16	0	0	–
Frigerio et al. (168)	Italy	2011	Patients scheduled to have first THA/TKA	MELISA®	20	0	0	–
			One year after implantation	MELISA®	20	0	0	Content of implant NR
Vernes et al. (169)	Hungary	2013	Controls with no implant	LTT, cytokine release, LMI	7	0	0	Metal-induced reactivity was considered when all three assays showed significant change. Patch tests gave negative results at each time point
			Patients scheduled for Ti-containing THA	LTT, cytokine release, LMI	40	0, 0, and 0	0	–
			Three, 6, 12, 24 and 36 months after implantation	LTT, cytokine release, LMI	40	14, 12 (NR)	35, 30 (NR)	–
Thomas et al. (170)	Germany	2013	Healthy controls	LTT	14	0	0	–
			Patients with asymptomatic Ti dental implants	LTT	6	0	0	A reduced inflammatory cytokine response and concomitant IL-10 production from lymphocytes and monocytes were found in this group

AN, avascular necrosis; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; LMI, lymphocyte migration inhibition test; MELISA®, memory lymphocyte immunostimulation assay; MHS, metal hypersensitivity; MIF, migration inhibitory factor; NR, not reported; OA, osteoarthritis; RA, rheumatoid arthritis; THA, total hip arthroplasty; TJR, total knee arthroplasty. The stimulation index (SI) is defined as the quotient of test counts per minute (cpm) and average negative control (background) cpm. An SI of ≥ 3 is considered to be positive (i.e. indicative of specific sensitization), an SI of ≥ 2 but < 3 ambiguous, and an SI of < 2 negative.

Table 5. Case reports of suspected titanium hypersensitivity

Author	Country	Year	Exposure	Clinical presentation	Test for Ti hypersensitivity	Time to reaction	Comment
Patch test							
Brun and Hunziker (171)	Switzerland	1980	Ti-containing pacemaker	Dermatitis of overlying skin	Patch tests with Ti metal and TiCl ₄ negative	1–2 years	–
Peters et al. (172)	USA	1984	Ti-containing pacemaker x 4	Pruritus, redness and swelling of overlying skin	Positive patch test reaction to Ti metal disc and artificial sweat	6 weeks to 17 months	A similar reaction occurred after implantation of a polyethylene-coated device
Abdallah et al. (173)	USA	1994	Pacemaker x 3	Vesicular lesions on overlying skin	Positive patch test reaction to Ti	3–8 months	Specific salt and formulation used for patch test NR
Viraben et al. (174)	France	1995	Ti-containing pacemaker	Dermatitis with granulomatous reaction of overlying skin and spread to the back and abdomen	Negative patch test results with TiO ₂ and pacemaker Ti component	2 months	Regression with topical steroids and spontaneous resolution
Ishii et al. (175)	Japan	2006	Ti-containing pacemaker x 2	Dermatitis with granulomatous reaction of overlying skin	Positive patch test reaction to pure Ti metal	Years	No recurrence of dermatitis after reimplantation with polytetrafluoroethylene-coated pacemaker
Nawaz and Wall (176)	USA	2007	Ti-containing spinal bioprosthesis	DRESS syndrome	Positive patch test reaction to Ti and Ni	1–2 months	Implant not removed. Regression of symptoms with slowly tapered prednisone over 6 months. Specific salt and formulation used for patch test NR
Syðurra et al. (177)	Switzerland	2009	Ti-containing pacemaker x 2	Aseptic pyoderma of overlying skin	Negative patch test results with Ti can from pacemaker	Days	No recurrence after reimplantation with gold-coated pacemaker
van Opstal and Verheyden (178)	Belgium	2011	Ti tibial baseplate in TKA x 2	Painful swelling of the knee, aseptic loosening and dermatitis of overlying skin	Negative patch test results with Ti	6 weeks	Resolution after revision with a non-Ti-containing implant. Specific salt and formulation used for patch test NR
Goto et al. (179)	Japan	2013	Ti suture anchors in rotator cuff	Erythema and itching on face, trunk, and hands	Negative patch test results with Ti	3 weeks	Resolution after replacement with non-Ti-containing sutures. Specific salt and formulation used for patch test NR
Sakamoto et al. (180)	Japan	2014	Ti bars after surgery for pectus excavatum	High fever, eosinophilia, and pleural effusion with high eosinophil count	Negative patch test result with Ti alloy plate pre-implantation	A few days	Oral steroid therapy (prednisone 20 mg/day) was initiated, with quick improvement of symptoms. Afterwards, prednisone was slowly tapered and continued until the bars were removed 2 years later
Wang et al. (181)	China	2015	Osteotomy with Ti screws	Persistent low-grade fever for >7 months + itching and erythema on contact with a watch or metal buttons	Positive patch test reaction to Ti miniplant	7 days	Extensive laboratory and radiological tests gave unremarkable results, and there was no effect of antibiotics. Ti screws were removed and, after several days, the patient's temperature had returned to normal. The patient was followed up for >1 year, and his body temperature remained <37 °C during this time
In vitro test							
Tamai et al. (197)	Japan	2001	Ti surgical clips	Exacerbation of atopic dermatitis	Positive BDORT** ^a . Patch test and LTT not possible	2 months	The patient's medical history showed worsened dermatitis when the patient used cosmetics containing high levels of TiO ₂ . Twelve months after removal of the clips, the atopic dermatitis had not completely resolved, so a possible allergy to dental materials was investigated
Thomas et al. (144)	Germany	2006	Ti miniplant and screws, osteosynthesis of metacarpal fracture	Hand eczema and failure of a hand fracture to heal. Eczema extended to the other hand and forearms	Positive LTT. Negative patch test	A few weeks	Resolution of dermatitis and healing of fracture after removal of implants. LTT normalized

Table 5. Continued

Author	Country	Year	Exposure	Clinical presentation	Test for Ti hypersensitivity	Time to reaction	Comment
Egusa et al. (198)	Japan	2008	Ti dental implants	Persistent facial dermatitis	Positive LTT. Patch test NP	1 week	Complete resolution after removal of implants
Belchalek et al. (199)	Czech Republic	2013	NITINOL foramen ovale occluder	Generalized exanthema	Positive MELISA®. Also mildly positive MELISA® and positive patch test reaction to Ni	3 days	The patient also started to report contact dermatitis caused by Ti earrings, and described burning sensations in the oral cavity after consumption of certain foods. Complete resolution after removal of implant
Ko et al. (200)	Japan	2014	Ti dental implants	Neck dermatitis	Negative LST pre-implantation. Positive LST post-implantation	9 months	Complete resolution after removal of implants
No test performed							
Verbov (201)	England	1985	Ti-containing pacemaker x 4	Aseptic pyoderma of overlying skin	NP	Months	–
Mitchell et al. (202)	USA	1990	Ti dental implants	Gingival hyperplasia, 2 patients	NP	NK	Complete resolution of symptoms after replacement with gold implant
High et al. (203)	USA	2006	Ti-containing ear piercings	Granulomatous dermatitis	NP	Shortly after	Lesions also contained particles of aluminium and vanadium
du Preez et al. (204)	USA	2007	Ti dental implants	Chronic inflammatory response, fibrosis, and foreign body giant cell reaction	NP	NK	Resolution after implant removal

BDORT, bi-digital O-ring test; DRISSE, drug reaction with eosinophilia and systemic symptoms; LST, lymphocyte stimulation test; LTT, lymphocyte transformation test; MELISA®, memory lymphocyte immunostimulation assay; NK, not known; NP, not performed; NR, not reported; TKA, total knee arthroplasty.

allergy, and a large proportion of the published cases lack complete allergy work-up or have used insufficient tests and patch test preparations. It should also be emphasized that there are several reported cases of suspected Ti allergy that, after further investigations, were found or suspected to be attributable to other allergens or causes (136, 183–189).

As implant failure in dentistry is often unexplained, and Ti allergy is barely recognized in this field, some authors have suggested that this could just be the very tip of the iceberg, and advocate further awareness (6, 182, 190). Increased awareness might also be advisable when implanting Ti into children and young adults. Children with severe scoliosis often receive Ti and Co–Cr instrumentation, which is prone to releasing large amounts of Ti for many years. So far, no cases have been reported but, not infrequently, patients are re-operated on because of pain, implant failure, or suspected infection.

We have not been able to find any reports of allergic reactions to Ti in personal care products or to TiO₂ in general. However, it has been proposed that TiO₂ present in cosmetics and physical blocker sunscreens adsorbs gold released from jewellery. Therefore, although many patients will not show dermatitis at the site of contact with gold jewellery, TiO₂ may result in chafing and the liberation of fine gold particulates that could travel and come into contact with the face and eyelids. Therefore, when, for example, eyelid dermatitis is investigated, the high prevalence of gold allergy/positive patch test reactions and possible interaction with TiO₂ justifies a trial of avoidance of gold jewellery or TiO₂, even when patients do not have dermatitis at the site of primary contact (191).

Conclusion

Exposure to Ti from both implants and from personal care products is common, but reports on clinical allergy are rare. With regard to implants, pure Ti, and alloys, it is evident that Ti ions are released from the surface and sometimes into distant tissue. However, evidence for the possible toxic effects and risk of allergic reactions is weak. TiO₂, the most widely used oxide, is much more stable than pure Ti and the alloys used in implants, and it seems very important for this difference to be taken into account when future studies are conducted or analysed. The prevalence of Ti allergy is difficult to assess, because of uncertainty in the methods of detection, but we estimate it to be very low. This is supported by the mounting evidence that Ti and TiO₂ (often as NPs) do not penetrate through intact skin. Patch testing with the currently available Ti and TiO₂ formulations is of no value in clinical practice, and, at present, there is no reliable way to test for Ti allergy.

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