Cytotoxic effects of submicron and nano-scale titanium debris released from dental implants: a systematic review

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Abstract

Objective: The objective of this study was to perform a systematic review on the toxic effect of submicron and nano-scale commercially pure titanium (cp Ti) debris on cells of peri-implant tissues.

Materials and Methods: A systematic review was carried out on the PUBMED electronic platform using the following keywords: Ti "OR" titanium "AND" dental implants "AND" nanoparticles "OR" nano-scale debris "OR" nanometric debris "AND" osteoblasts "OR "Cytotoxicity" OR "mutagenic"

Results: Titanium nanoparticles in submicron- and nano-scale altered the behavior of cells in culture medium. An inflammatory response was triggered by macrophages, fibroblasts, osteoblasts, mesenchymal cells, and odontoblasts as indicated by the detection of several inflammatory mediators: IL-6, IL-1β, TNF-α and PGE2. The formation of a rich bioactive complex composed of calcium and phosphorus on titanium nanoparticles allowed the binding to proteins leading the cell internalization phenomenon. The nano-particles induced mutagenic and carcinogenic effects into the cells.

Conclusions: The cytotoxic effect of debris released from dental implants depends on the size, concentrations, and chemical composition of the particles. A high concentration of particles on nanometric scale intensifies the inflammatory responses with mutagenic potential of the surrounding cells.

Clinical relevance: Titanium (Ti) ions and debris have been detected in peri-implant tissues with different sizes and forms. The presence of metallic debris at peri-implant tissues also stimulates the migration of immune cells and inflamatory reactions. Cp Ti and TiO_2 micro- and nano-scale particles can reach the blood stream, accumulating in lungs, liver, spleen, and bone marrow.

Key words: cytotoxicity; titanium; debris; nanoparticles; genotoxicity; systematic review.

1.Introduction

The long-term success of the dental implants has been reported in previous studies although approximately 70% of the failures in implant and supported prosthetics have occurred due to inflammatory reactions [1–3]. Inflammatory reactions confined to the soft tissues are named mucositis while the progression of the inflammatory reactions whereby bone loss is known as peri-implantitis [2–5]. Such inflammatory reactions have multiple etiologic factors attributed to biological or physicochemical factors, such as: peri-implant inflammations leading to bone loss, degradation of structural materials and connections, implant design, surgical and prosthetic complications; and patient-specific conditions [3, 4, 6]. Inflammatory peri-implant tissue diseases originating from biofilm accumulation containing pathogenic microorganisms can cause bone resorption and loss of the implant [3, 5]. Progressive osteolysis and consequent bone injury level depend on the host response that have been discussed concerning the patient-related health conditions such diabetes or osteopenia [2, 7]. Furthermore, it has become more clear over the past years that the presence of metallic debris (i.e., titanium, aluminum, vanadium) at peri-implant tissues also stimulates the migration of immune cells and inflamatory reactions [8–11]. Titanium (Ti) ions and debris have been detected in peri-implant tissues with different sizes and morphological features potentially contributing to chronic inflammatory reactions and peri-implant bone loss [12–14]. The accumulation of Ti ions and debris takes place due to the degradation of abutment and implant surfaces and implant-abutment connections [10, 15, 16]. Also, therapeutic procedures by using acidic substances and fluorides or mechanical debridement

(implantoplasty) can corrode the titanium-based surfaces and induce the release of Ti ions [9, 17].

The degradation of the implant-abutment connection causes an increase in microgap size, materials loss and mechanical instability [10, 15]. The design of the implant systems plays an important role in the maintenance of the implant-abutment microgap and mechanical stability[18–20]. Another factor that can influence stability is the type of titanium Commercially pure titanium (cp Ti) grade IV and Ti13Zr are the mostly used for manufacturing endosseous implants while Ti6Al4V is used for abutments [21]. The elastic modulus of the cp Ti and Ti13Zr is approximately 100-110 GPa while the Ti6Al4V has an elastic modulus of around 150 GPa which are significantly higher compared to the bone of (10-30 GPa)[22]. Nevertheless, the tensile strength of both types of titanium have sufficient tensile strength values to withstand mechanical stresses in the oral cavity (cpTi grade IV at 550 MPa, Ti13Zr and Ti6Al4V at about 940-1000 MPa) [22, 23].

The chemical composition of titanium-based materials is responsible for their chemical stability (i.e. resistance to corrosion) in different media, especially in contact with acidic and reactive substances [24, 25]. These properties are determined by the thin layer of titanium oxide that forms spontaneously on the surface of the cp Ti and Ti alloys when exposed to the room environment due to the high affinity of Ti for oxygen. Such passive film, composed mainly of TiO_2 has a thickness of approximately 1.5-10 nm [24, 25], which protects the titanium from aggressive substances such as lactic acid, citric acid, hydrofluoric acid (HF), microbial metabolites, and fluorides [26, 27]. Nevertheless, the thin TiO_2 layer can be altered and damaged due to variations in temperature, pH, oxygen levels, bacteria metabolites, and dietary. Fluoride-containing varnishes, gels, and oral rinses which are commonly used to prevent dental caries and tooth demineralization, induce a progressive destruction of the

titanium oxide layer [9, 21]. A solution containing more than 30 ppm HF may promote the localized corrosion of titanium [28]. The chemical treatment of the implants with citric acid as a treatment for peri-implantitis treatment is also considered as an aggressive process that damages the surface of the implant and abutment [17, 21]. The destruction of the titanium passive film cause the release of Ti, Al, V ions and titanium-based debris at a micro- and nano-scale [9, 26].

The metallic debris released from titanium implants consists of several chemical and morphological aspects, including organometallic complexes, free metallic ions, and inorganic metal oxides such as cp Ti or TiO_2 particles[29, 30]. Cp Ti and TiO_2 submicron and nanoparticles are widely used to provide whiteness and opacity to products such as adhesives, toothpastes, and sunscreen, and other cosmetics. In 1999, TiO_2 was considered as a group 3 carcinogen, meaning that it has no carcinogenic effect on humans [29]. In 2006, the International Agency for Research on Cancer (IARC) classified TiO_2 nanoparticles as an IARC Group 2B carcinogen ''possibly carcinogenic to humans'' [31]. Also, several previous studies revealed the local and systemic toxic effects of TiO_2 nano-particles [32–34]. Cp Ti and TiO_2 micro- and nano-scale particles can reach the blood stream, accumulating in lungs, liver, spleen, and bone marrow. That contributes to the development of nanoparticle-associated diseases in the respiratory or cardiovascular systems, which can even lead even to malignant tumor [35, 36].

Considering the findings data on the detrimental effects of titanium debris, it was hypothesized that micro- and nano-scale titanium particles induce inflammatory reactions and DNA damage on cells of peri-implant tissues. Thus, the main aim of this study was to perform a systematic review on the toxic effect of submicron and nano-scale titanium debris

released from dental implants in contact with peri-implant tissues as a consequence of wear and corrosion phenomena.

2.Method

A bibliographic review was performed on PUBMED (via National Library of Medicine) using the following search terms: “Ti” OR “titanium” AND “dental implants” AND “nanoparticles” OR “nano-scale debris” OR “nanometric debris” AND “osteoblasts” OR “cytotoxicity” OR “mutagenic” OR “apoptosis”. A manual search of the reference lists in the selected articles was also performed. The inclusion criteria encompassed articles published in the English language, until January 16th, 2020, reporting the effect of nano-scale titanium particles as released from dental implants on the toxicity and damage of osteoblasts. The eligibility inclusion criteria used for article searches also involved: cell culture assays; *in vitro* characterization; meta-analyses; randomized controlled trials; animal assays; and prospective cohort studies. Two of the authors (JCMS, RMH) independently analyzed the titles and abstracts of the retrieved, potentially relevant articles meeting the inclusion criteria. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager. Selected articles were individually read and analyzed concerning the purpose of this study. The following variables were collected for this review: authors’ names, journal, publication year, aims, content and size of titanium nanoparticles; study design, cell culture methods, and toxicity effects on osteoblasts.

3.Results

The initial search in the available database yielded a total of 186 articles of which 46 duplicate articles were eliminated. Of the remaining 140 articles, the titles and abstracts were read seeking concordance with the inclusion criteria of the present study and therefore 22 studies were discarded after because they were related only considering TiO_2 or titanium alloys. The evaluation of titles and abstracts resulted in the selection of 14 potentially review articles as shown in Figure 1.

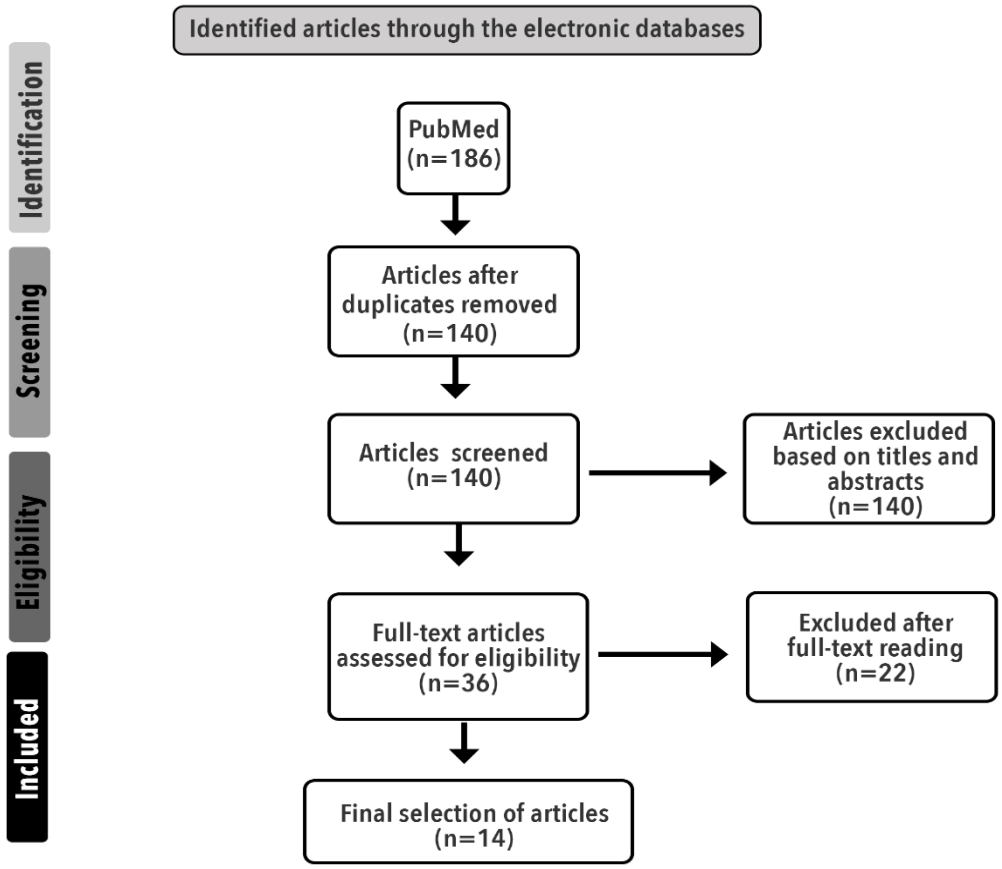


Figure 1. Flow diagram of the search strategy used in this study.

Of the 14 articles included in this review, three (21.42%) *in vitro* studies evaluated the toxicity induced by titanium particles in contact with osteoblast [37–39], of which two assessed human cell lines [37, 39]. Three *in vitro* studies (21.42%) investigated the toxic effect of

titanium debris on human fibroblast [12, 37, 40], whereas one of them was in tissues from biopsies[40]. One article evaluated the toxic effects of titanium debris released from dental implants on oral epithelial cells [41], while another one investigated the toxicity of titanium particles on periodontal ligament cells which were genetically modified with lentiviral gene transfer of human telomerase reverse transcriptase cells [42]. One *in vitro* study examined the human mesenchymal stem cells cytotoxicity upon exposure to submicron particles of titanium[43]. One *in vivo* study investigated the effects of titanium wear debris on the nutritive perfusion and leukocytic response in skeletal muscle of Syrian golden hamsters [44], while three studies (21.42%) performed in human participants reported the inflammatory reaction of macrophages and monocytes against titanium particles [45–47] of which one used tissues harvested from biopsies [46]. Only, two articles (14.28%) described the mechanism of debris released from dental implants[48, 49], in which one investigated the surface characterization of titanium healing abutments before and after placement in patients[48], and the other one reported the in vitro effect of ultrasonic scalers on titanium surfaces[49].

The main outcomes can be drawn as follow:

- The size of nanoparticles used in the different studies ranged from 20 up 100 nm[42, 45], while other articles reported micrometric particles ranging from 0.25 up to 43 µm[12, 37–39, 42–47];
- Several proteins were involved in the toxicity induced by titanium particles according to the cell culture. An *in vitro* study on macrophages revealed a high level of inflammatory mediators such as MyD88, TRIF and NF-kB in the presence of titanium particles size (≤ 20 µm) [37]. Other *vitro* studies performed on human monocytes and macrophages, titanium particles with size ranging from (0.25 up to 7 µm) were able to induce a pro-inflammatory response, characterized by the increased expression and

release of transcripts and proteins linked to TNF-α, IL-1β and IL-6 cytokines [45–47]. Protein expression was higher for IL1-β at all time points when compared to TNF-α and IL-6. IL-1β is the cytokine expressed to start the osteolytic process and plays an important role to TNF-α expression that modulates RANK-L production, leading to bone resorption[45–47];

- In *in vitro* cultured osteoblast-containing medium, metallic wear debris (0.90-1.50 µm in size) resulted in changes in the expression of the markers of the ER stress apoptotic pathway, namely GRP78 and GADD153, and downstream caspase cascades, ultimately leading to cell apoptosis[38]. In another *vitro* study it was demonstrated that Ti particles (size 0.488 µm) caused up-regulation of the zinc finger protein 467 (ZFP467), which is a regulator of osteoblast and adipocyte indicating that a switch between osteogenic and adipogenic phenotypes had begun[12];
- Relatively to the toxic effect of titanium particles with size ranging from (0.25 up to <20 µm) in contact with fibroblasts, the data showed high expression of inflammatory mediators, such as interleukin 6 (IL6) and IL1B, but also an increase in the anti-inflammatory one such as IL10. In addition to chemokine (C-X-C motif) ligand 2 (CXCL2), conversely (CXCL5) showed lower transcript levels[12, 37, 39]. Also, high levels of protein kinase C beta (PKCB), which is involved in adipogenesis caused by titanium particles of size (0.488 µm) [12], and higher levels of phospho-FAK protein were detected [40]. Another study was performed on normal oral keratinocyte spontaneously immortalized cells (NOK-SI) to evaluate if titanium debris release from different implant surfaces were biocompatible with oral epithelial cells or caused DNA damage. The results demonstrated that titanium particles from certain implant

systems were able to activate CHK2 and trigger the recruitment of BRCA1, which are markers of DNA damage and genomic instability [41];

- One article related to this topic, carried out a comparison between the cytotoxicity and cellular uptake of three different titanium particles by periodontal ligament cell which were genetically modified via lentiviral gene transfer with human telomerase reverse transcriptase cells (PDL-hTERT). The results demonstrated that cp Ti particles with a size of 20 nm and 250 nm induced higher cellular uptake efficiency and higher toxic potential than microparticles and therefore nanoparticles were found in the nucleus [42];
- Another *vitro* study described how titanium particles with a submicron size (0.380 μm) in culture of human Mesenchymal stem cells (hMSCs) were able to affect adversely the cell viability through the induction of apoptosis, eliciting increased expression of the tumor suppressor proteins p53 and p73 in a dose and time dependent manner oin human mesenchymal stem cells (hMSCs) [43];
- An *in vivo* study in skeletal muscle of golden hamsters showed that titanium particles with submicron size (0.6 μm) caused an acute inflammatory process mediated by polymorphonuclear leukocytes with recruitment of leukocytes [44]. In a progressive way, that was associated to chronic inflammatory reactions.

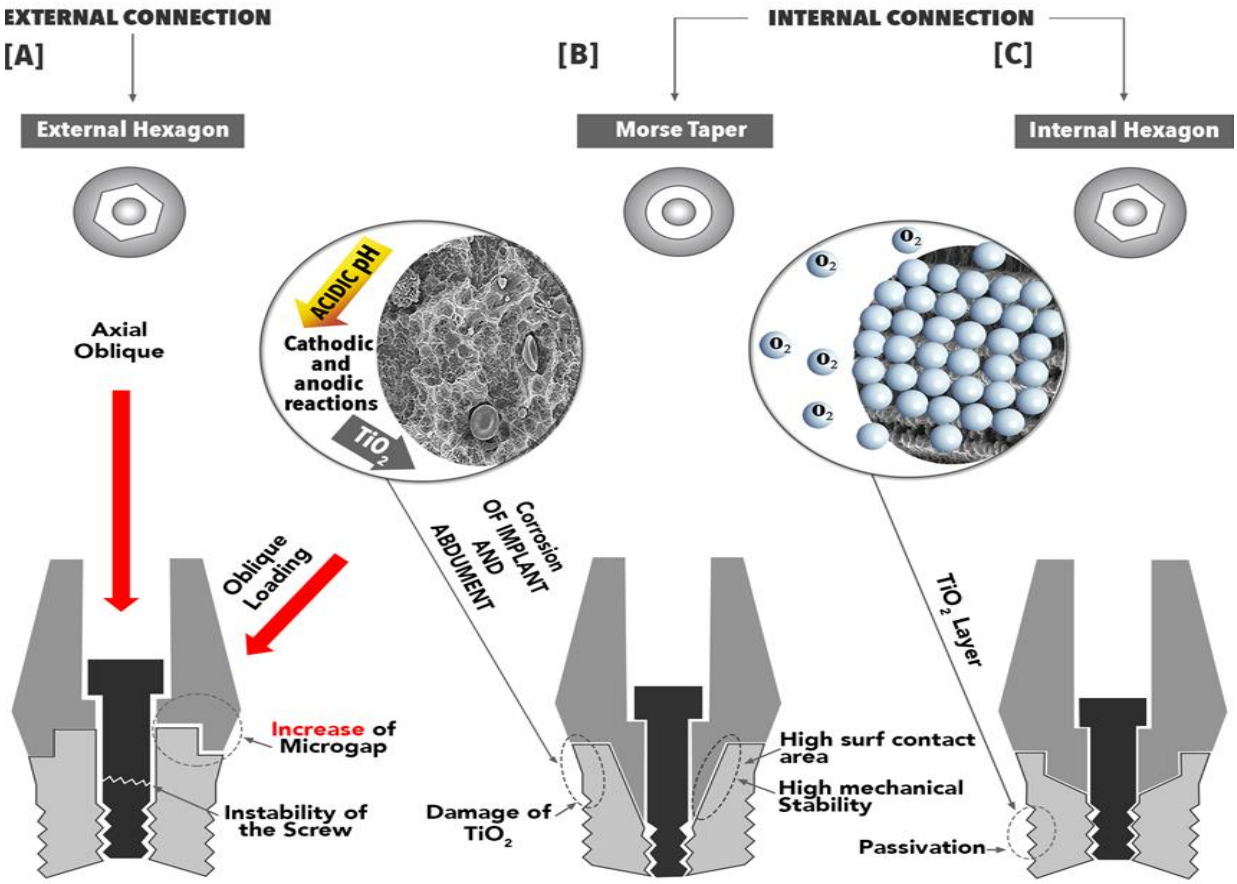
4. Discussion

The findings reported in the retrieved articles validate the hypothesis of the present study. Indeed, the size and content of titanium nano-debris released from implants negatively affect the osteogenic cell behavior, inflammatory response, and peri-implant tissues' health state.

Parameters related to titanium, the surface, the release of debris, and toxicity are discussed as follow.

4.1. Titanium implants and abutments

Most endosseous implant systems for oral rehabilitation consist of a set of two major components including the implant and abutment for prosthetic rehabilitation. However, the connections between these components are subject to chemical degradation by corrosion and abrasion wear due to micro movements from mastication forces in contact with oral solutions [10, 15]. The degradation of the implant-abutment connection causes an increase in microgap size, materials loss, mechanical instability, and release of implant debris to the surrounding tissues [10, 21]. The design of the implant systems plays an important role in the maintenance of implant-abutment microgap and mechanical stability [18–20]. The main types



of implant-abutment connections are hexagon (external, internal) or Morse taper, as shown in Figure 2.

Figure 2. Schematics of different titanium implant-abutment connections.

The external hexagon implant-abutment connection reveals disadvantages concerning the loosening of the abutment screw and increase in microgap size leading to lateral or oblique loading during mastication [15, 50]. The mechanical instability in external hexagon implant-abutment connections has been reported in clinical studies on the abutment torque inspection [51, 52]. The instability of the implant-abutment connections results in oblique or horizontal loads at higher magnitude when compared to axial loading. The internal implant-abutment connection emerged to solve the mechanical instability reported in may studies, thus seeking long-term mechanical stability, decrease in microgaps' size, and inhibition of biofilm accumulation [18, 19]. Internal connections comprise internal hexagon and Morse taper design (Fig. 2) though the Morse taper provides more intimate implant-abutment contact [20].

Commercial pure titanium (cp-Ti) grade IV has been considered the first choice for manufacturing dental implants while Ti6Al4V alloys are used to produce abutments [53]. Titanium and its alloys has a lower density (around 4.7 g/cm3) when compared to 7.9 g/cm3 stainless steel (AiSi 316 L) 316 and 8.3 g/cm^3 Cobalt Chromium-Molybdenum (CoCr-Mo)[22]. The tensile strength of cp Ti grade IV is lower (~550 MPa) when compared to that (950 MPa) recorded for Ti6Al4V [9] . However, the relationship of density and strength is more than enough for application as implant-abutment connections [53]. The mechanical properties of titanium were improved by adding metal elements such as aluminum (Al),

vanadium (V), niobium (Nb), and zirconium (Zr). CpTi consists of the α phase (hexagonal close-packed; HCP) while Ti6Al4V consists of both the α and β (body-centered cubic; BCC) phases stabilized by Al and V [22, 53].

The high resistance to corrosion and biocompatibility of titanium-based materials is dependent on a surface thin film composed of titanium oxide, mainly TiO_2 [24, 25]. Such stable titanium oxide film is spontaneously formed at the titanium implant surface within a thickness between 1.5-10 nm when exposed to ambient air due to the high affinity of Ti for oxygen, known as passivation [24, 25]. Nevertheless, the titanium oxide layer can be altered and damaged by continuous corrosion and wear [54] (Figure 2).

Recent studies have reported local and systemic adverse biological reactions induced by titanium debris released from CpTi and Ti6Al4V surfaces [28]. Aluminum (Al) and Vanadium (V) are considered toxic elements, and, therefore. Vanadium has revealed a mutagenic potential [55]. For this reason, alternative titanium alloys have been developed involving Nb, Zr, and tantalum (Ta) as β phase stabilizing elements, causing fewer toxic effects, and maintaining the corrosion resistance and mechanical properties [56].

4.2 Release of titanium debris from dental implants

The presence of titanium debris in the oral tissues begins from the moment of placement and friction of the implant into the surgical bone site [48, 57]. The rotating procedure on placing the implant into the bone causes the wear of the titanium oxide film and bulk material[9, 17]. Additionally, the surfaces of the implant connection and abutment will be continuously exposed to the oral cavity, as illustrated in Figure 3. In this way, the exposed surfaces are susceptible to the chemical effect of the acidic substances (e.g. lactic acid) from the oral fluids or microbial metabolism [21, 54, 58]. Previous studies have reported an acceleration in the

degradation of the protective titanium oxide thin layer in the presence of lactic acid, hydrogen peroxide, citric acid, artificial saliva, or fluoride solutions [21, 54, 58].

Corrosion and wear are considered an inherent process in the oral cavity since the chemical reactivity of titanium metallic materials such as titanium increases in contact with oral or therapeutic substances [59]. The chemical reactivity of titanium significantly increased when immersed in therapeutic fluoride solutions or gels that are used by dentists and patients [9]. In fact, high concentrations of F^- promote an association between, H^+ and F^- ions, forming hydrofluoric acid (HF), which is corrosive to titanium [60]. Even lower concentration of F^- in acidic environment influences the corrosion resistance of titanium [59]. Corrosion occurs in contact with acidic substances from dietary, bacteria metabolism (e.g. lactic acid), and therapeutic treatment (e.g. fluorides, citric acid) [60, 61], as illustrated in Figure 3. Wear starts on placement of abutment or healing caps over titanium surface connections due to the friction of the contacting surfaces (Figure 3) [15, 50]. On mastication loading, wear can take place by abrasion caused from the micro-movements on implant, abutment, and prosthetic contacting surfaces [62]. The wear of titanium surfaces can remove the protective titanium oxide film and expose the bulk titanium to the acidic environment [21, 54, 58]. In this case, the synergistic interaction between physical and chemical processes (wear and corrosion) can increase the degradation of the titanium surfaces (Figure 2 and 3) and increase the release of titanium debris and ions to the surrounding tissues [61].

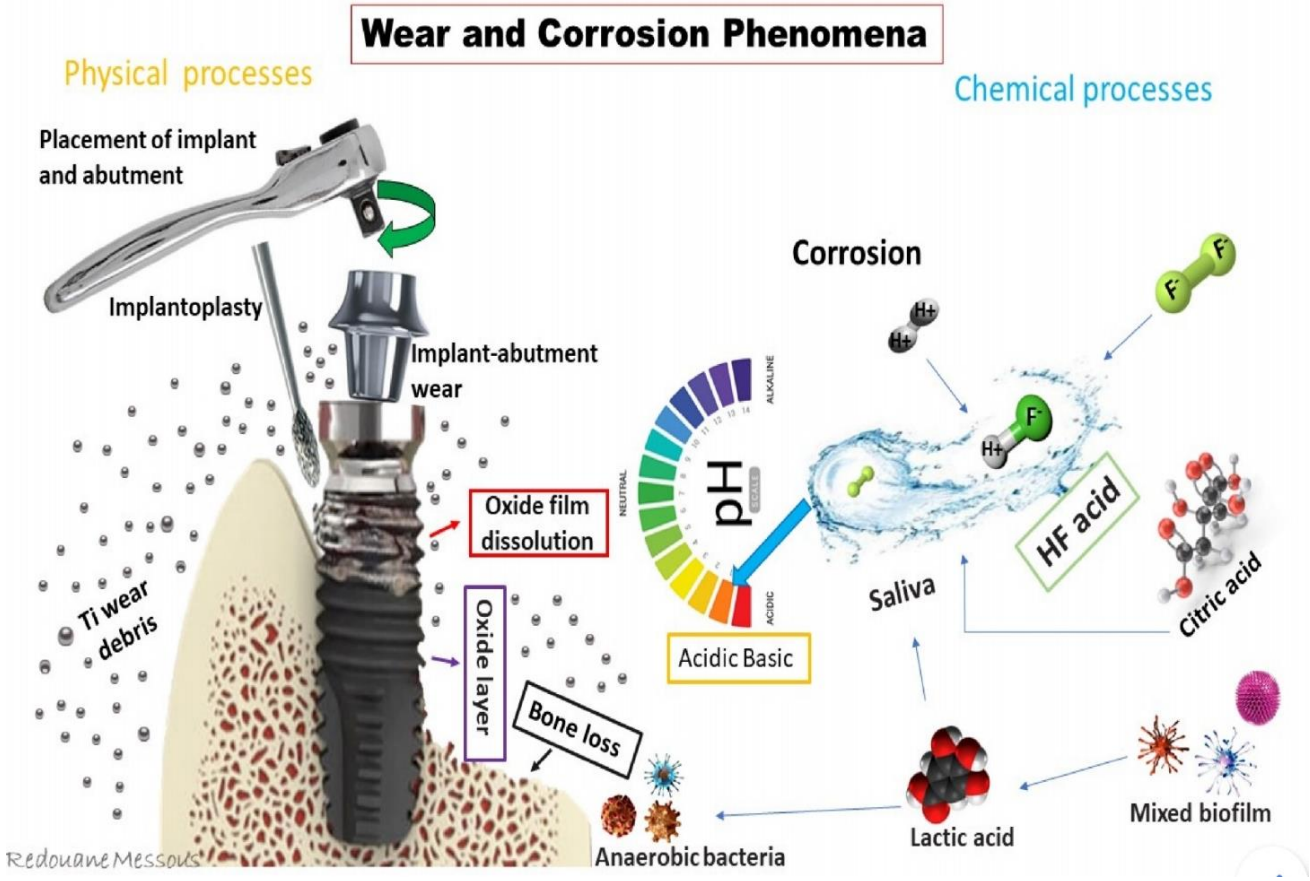


Figure 3. Degradation pathways of implant-abutment connections and consequent release of debris.

The fitting of the abutment and implant parts is crucial to decrease the micro-movements and metallic debris [62]. The degradation of titanium-based surfaces leads to the materials loss and release of ions (Ti, Al, V) and metallic debris ranging from macro- to nano-scale size into the surrounding tissues [63, 64]. In a cross-sectional study, the level of Ti in the peri-implant tissue was examined in 200 biopsies of which 160 from patients affected by peri-implantitis and 40 from patients healthy. The histological analysis showed presence of Ti and other metals (e.g. Zn, Al, Cu, and Ru) inside all samples affected by peri-implantitis [12]. It was demonstrated that the interaction of Anatase Titanium Dioxide nanoparticles with calcium (Ca), phosphorous (P) and hydroxyapatite (Hap) deriving from the medium culture due to the

high reactivity of Anatase Titanium Dioxide. In FBS medium, protein is adsorbed on the surfaces of the titanium nanoparticles resulting in a bio-complex including serum albumin and other glycoproteins (ALB protein and Alpha 2HS) [30]. The role of the proteins is creating an initial nano-bio interface that undergoes dynamic alterations as particles traffics onto or into cells [30]. Previous studies showed that metallic particles released from dental implants can accumulate in the surrounding tissues [9, 65], or even spread systemically, as reported in previous studies [66, 67], contributing to the chronic inflammatory state in peri-implant diseases [68, 69].

Physical and chemical decontamination methods in the treatment of peri-implantitis can also induce the release of titanium debris to the surrounding tissues [13, 64]. Previous studies showed that sonic and ultrasonic scalers with metal tips cause considerable changes onto implant surfaces [49, 64]. Implantoplasty should be highlighted as an aggressive process of release of titanium debris from dental implants (Fig.3). In peri-implantitis treatment, implantoplasty is also used by several dental practitioners as a mechanical therapeutic method for decontamination of dental implants by removing titanium layers from the rough implant surfaces [64] (Figure 3). Nevertheless, such physical process results in the release of a high content of titanium debris and ions to the surgical bone site and connective tissues[17]. Also, the debris can be released from the burs used for implantoplasty [9].

4.3 The toxic effect of titanium particles on cells and tissues

Submicron- and nano-scale debris released from dental implants as a result of degradation, are considered as foreign bodies which activate the human immune system. Several inflammatory mediators and cytotoxins associated with peri-implantitis disease and bone resorption are involved [37, 40, 46], as seen in Figure 4. As a first defense line of the immune

system, the most representative leukocytes (54-65%) with the function of phagocytosis are neutrophils and macrophages [70].

In fact, cytotoxicity depends on the size, chemical composition, and content of metallic particles [70, 71]. Studies showed that neutrophils phagocytized Ti particles only when the particles were smaller than the cells (~5 μm)[70], while macrophages revealed a more complex function [47]. Monocytes and macrophages have a specific relationship with individual cytokines and respond to various cytokines secreted after neutrophils reaction with foreign bodies [70]. Titanium particles ranging from 0.25 up to 7 μm in contact with macrophages could induce a pro-inflammatory response with an increase in expression of transcripts and proteins such as TNF-α, IL-1β, and IL-6 cytokines [45–47]. Sub-micron and nanoparticles caused the highest gene expression for all cytokines [72] probably because the cell internalization of the titanium particles [73]. Therefore, micro and nano-particles of titanium induce an increase in secretion of IL-1β from macrophages leading to bone resorption by osteoclast activity via activation of RANKL expression [74, 75]. Titanium micro-scale particles size (≤ 20 μm) also induced the release of IL-6 and TNF-α from fibroblasts [76]. An in vitro study with titanium and TiO_2 particles size below 7 μm also revealed an increase in the amount of IL-6 and PGE2 produced by osteoblast [39, 68]. Thus, titanium particles promote a dual effect: first, the particles induce an inefficient bone formation due to the inhibition of osteoblast proliferation; and second, particles increased bone resorption by IL-6 osteoblast-mediated osteoclastogenesis [77].

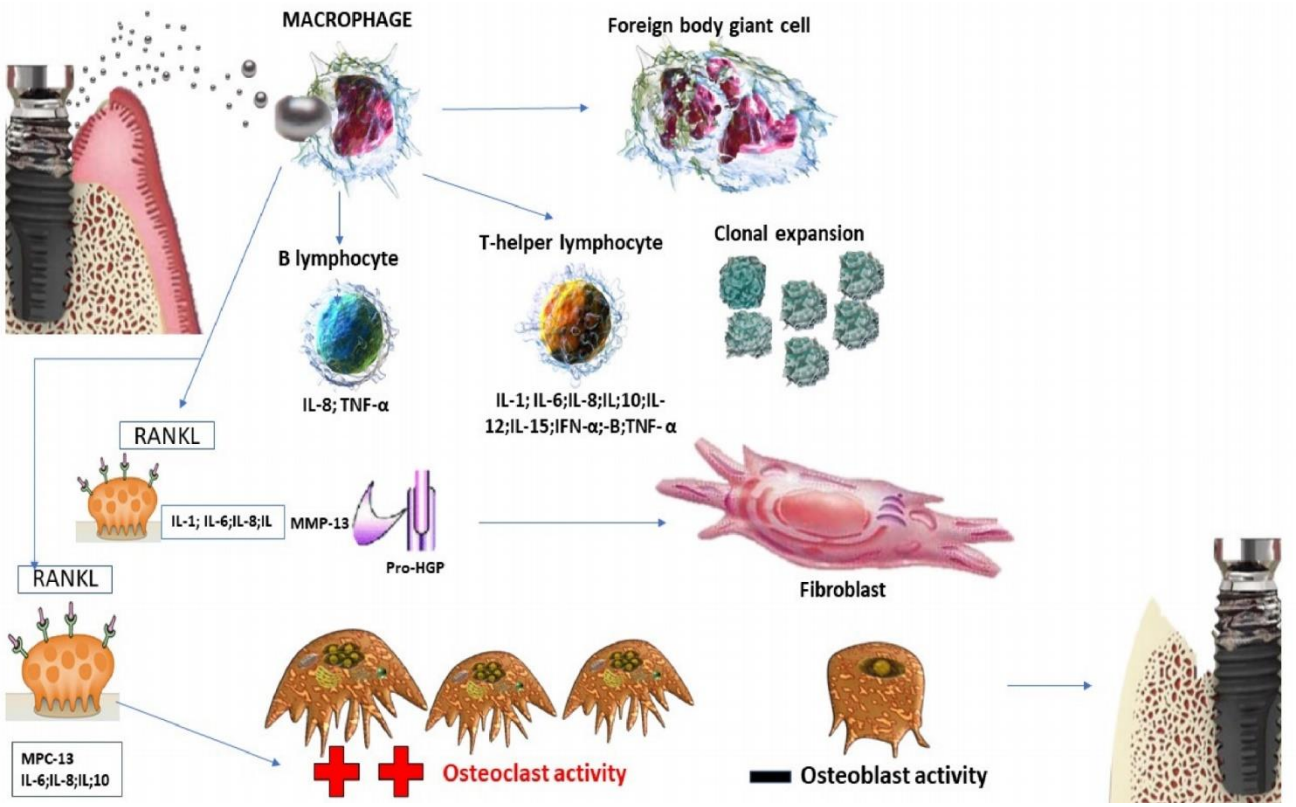


Figure 4. Schematics of the effect of cp Ti debris on surrounding cells and tissues.

An *in vitro* study assessed wear debris harvested from patients with aseptic joint loosening to ensure that the experiments more closely resembled the biological *in vivo* response. Titanium particles at a size of 0.90-1.50 µm were placed in contact with primary osteoblast from rats [38]. The results indicated that reactive oxygen species (ROS) generation induced by metallic wear debris triggered endoplasmic reticulum (ER) stresses, mitochondrial dysfunction, and downstream caspase cascades, leading to cell apoptosis and consequently suppressing bone formation around the prosthesis [38].

In the presence of titanium particles, the inflammatory response can be progressive since a strong osteoclastogenesis process takes place due to the activation of mature osteoclast from macrophages [13, 77]. An *in vivo* study in skeletal muscle of golden hamsters showed that titanium particles (0.6 µm) caused an acute inflammatory process mediated by

polymorphonuclear leukocytes with recruitment of leukocytes might at some stage culminate in a chronic inflammatory reaction [44]. Another study analyzed the effect of 0.488 µm Ti particles against fibroblasts and mesenchymal stem cells and their subsequent distribution within peri-implant tissues. The results confirmed that titanium particles were embedded in all peri-implant tissue with different sizes and forms. Also, sub-micron particles were internalized within cells [12]. Submicron particles (0.380 µm) in contact with human mesenchymal stem cells (hMSCs) were able to affect adversely the cell viability by induction of apoptosis, eliciting increased expression of the tumor suppressor proteins p53 and p73 [43]. The adverse reaction magnitude was dependent on particle content and exposure time [43]. The production of matrix metalloproteinases (MMP) was also reported with a reduction in the expression of their inhibitor TIMP1, which can be associated with tissue destruction [12]. MMP are responsible for remodeling and degrading extracellular matrix molecules by cleaving components of cell-to-cell networking [78]. The role of PGE2 in titanium particles-induced osteoclastogenesis and in osteolysis has been shown in an *in vivo* mouse calvaria resorption model [79]. In previous studies, the increase in PGE2 have been detected in periprosthetic membranes of failed implants [80]. In addition to direct effects of titanium particles on osteoblasts, particle-mediated induction of PGE2 contributes to decreased levels of OPG[39], which is as a potent inhibitor of osteoclast differentiation and activation. That acts as a decoy receptor for RANKL and prevents its interaction with the cognate receptor RANK expressed in osteoclasts precursors[81].

5.Conclusions

The present review analyzed the *in vitro* and *in vivo* toxic effects of titanium debris released from dental implants in contact with different cell lines or tissues. Within the limitations of the *in vitro* and *in vivo* studies, the following outcomes can be drawn as follow:

- Different mechanical and chemical processes are involved in the release of macro-, micro-, submicron, and nano-scale titanium particles or ions from dental implants and abutments into the surrounding tissues. Submicron and nano-scale particles are not easy to be detected by conventional physicochemical methods and therefore they can be internalized by the cells regarding the size.
- Micro-scale cp Ti particles assessed in the selected articles had a size ranging from 0.3 up to 20 μm, while the size of nano-scale cp Ti particles varied from 20 up to 100 nm. In vitro studies assessed commercially metallic powders to avoid the bias of results although one study assessed particles with mean of 0.90-1.50 μm in size retrieved from patients.
- CpTi micro-scale particles in contact with culture of fibroblast induced an increase in the amount of IL-6, TNF-α and PGE_2 cytokines. That corresponds to a chronic inflammatory response. CpTi particles in contact with culture of osteoblast also induced increase of IL-6 and PGE_2 with a decrease of OPG, which can lead to bone resorption.
- Micro and nanoparticles of CpTi in contact with culture of monocytes and macrophages induce an increase of for TNF-α, IL-6 and IL-1β which stimulates bone resorption via activation of RANKL expression. The increase in the content of nano-scale cp Ti particles negatively affect the immune response.
- Acute inflammatory process mediated by polymorphonuclear leukocytes with recruitment of leukocytes was noticed in an animal study. Such inflammatory process

might result in a chronic inflammatory reaction that depends on the particle size and content.

- Despite the existing literature on the cytotoxicity of the debris released from the implants, few studies evaluate the cytotoxic effect of the CpTi particles on the peri-implant tissues, of which only one use particles in nano-scale, while the rest of the studies used particles in micro-scale. However, none of the studies investigated the mutagenic or genotoxic effect that CpTi particles can induce. More studies are required to evaluate the effects of the content, size and chemical composition of CpTi particles and also more *in vivo and in vitro* studies are required in different types of cells (macrophages, fibroblasts, osteoblasts, mesenchymal cells) to clarify the mechanisms involved in the cytotoxicity and genotoxicity of CpTi particles.

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References

1. Moraschini V, Poubel LADC, Ferreira VF, Barboza EDSP (2015) Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up

period of at least 10 years: A systematic review. Int J Oral Maxillofac Surg 44:377–388. https://doi.org/10.1016/j.ijom.2014.10.023

2. Albrektsson T, Buser D, Chen ST, et al (2012) Statements from the Estepona Consensus Meeting on Peri-implantitis, February 2-4, 2012. Clin. Implant Dent. Relat. Res. 14:781–782

3. Derks J, Tomasi C (2015) Peri-implant health and disease. A systematic review of current epidemiology. J Clin Periodontol 42:S158–S171. https://doi.org/10.1111/jcpe.12334

4. Kordbacheh Changi K, Finkelstein J, Papapanou PN (2019) Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a U.S. dental school. Clin Oral Implants Res 30:306–314. https://doi.org/10.1111/clr.13416

5. Lee CT, Huang YW, Zhu L, Weltman R (2017) Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. J Dent 62:1–12. https://doi.org/10.1016/j.jdent.2017.04.011

6. Patel K, Mardas N, Donos N (2013) Radiographic and clinical outcomes of implants placed in ridge preserved sites: a 12-month post-loading follow-up. Clin Oral Implants Res 24:599–605. https://doi.org/10.1111/j.1600-0501.2012.02500.x

7. Zhou W, Tangl S, Reich KM, et al (2019) The Influence of Type 2 Diabetes Mellitus on the Osseointegration of Titanium Implants With Different Surface Modifications-A Histomorphometric Study in High-Fat Diet/Low-Dose Streptozotocin-Treated Rats. Implant Dent 28:11–19. https://doi.org/10.1097/ID.0000000000000836

8. Fretwurst T, Nelson K, Tarnow DP, et al (2018) Is Metal Particle Release Associated with Peri-implant Bone Destruction? An Emerging Concept. J Dent Res 97:259–265. https://doi.org/10.1177/0022034517740560

9. Noronha Oliveira M, Schunemann WVH, Mathew MT, et al (2018) Can degradation products released from dental implants affect peri-implant tissues? J Periodontal Res 53:1–11. https://doi.org/10.1111/jre.12479

10. Apaza-Bedoya K, Tarce M, Benfatti CAMAM, et al (2017) Synergistic interactions between corrosion and wear at titanium-based dental implant connections: A scoping review. J Periodontal Res 52:1–9. https://doi.org/10.1111/jre.12469

11. Souza JCM, Apaza-Bedoya K, Benfatti CAM, et al (2020) A Comprehensive Review on the Corrosion Pathways of Titanium Dental Implants and Their Biological Adverse Effects. Met. 10

12. Bressan E, Ferroni L, Gardin C, et al (2019) Metal nanoparticles released from dental implant surfaces: Potential contribution to chronic inflammation and peri-implant bone loss. Materials (Basel) 12:. https://doi.org/10.3390/ma12122036

13. Berryman Z, Bridger L, Hussaini HM, et al (2019) Titanium particles: An emerging risk factor for peri-implant bone loss. Saudi Dent J. https://doi.org/10.1016/j.sdentj.2019.09.008

14. Wilson TG, Valderrama P, Burbano M, et al (2015) Foreign Bodies Associated With Peri-Implantitis Human Biopsies. J Periodontol 86:9–15. https://doi.org/10.1902/jop.2014.140363

15. Michalakis KX, Lino P, Muftu S, et al (2014) The effect of different implant-abutment connections on screw joint stability. J Oral Implantol 40:146–152. https://doi.org/10.1563/AAID-JOI-D-11-00032

16. Prado AM, Pereira J, Silva FS, et al (2017) Wear of Morse taper and external hexagon implant joints after abutment removal. J Mater Sci Mater Med 28:65. https://doi.org/10.1007/s10856-017-5879-6

17. Suárez-López Del Amo F, Garaicoa-Pazmiño C, Fretwurst T, et al (2018) Dental implants-associated release of titanium particles: A systematic review. Clin Oral Implants Res. https://doi.org/10.1111/clr.13372

18. Goiato MC, Pellizzer EP, da Silva EVF, et al (2015) Is the internal connection more efficient than external connection in mechanical, biological, and esthetical point of views? A systematic review. Oral Maxillofac Surg 19:229–242. https://doi.org/10.1007/s10006-015-0494-5

19. Saidin S, Abdul Kadir MR, Sulaiman E, Abu Kasim NH (2012) Effects of different implant-abutment connections on micromotion and stress distribution: Prediction of microgap formation. J Dent 40:467–474. https://doi.org/10.1016/j.jdent.2012.02.009

20. Macedo JP, Pereira J, Vahey BR, et al (2016) Morse taper dental implants and platform switching: The new paradigm in oral implantology. Eur J Dent 10:148–154. https://doi.org/10.4103/1305-7456.175677

21. Souza JCM, Henriques M, Teughels W, et al (2015) Wear and Corrosion Interactions on Titanium in Oral Environment: Literature Review. J Bio- Tribo-Corrosion 1:. https://doi.org/10.1007/s40735-015-0013-0

22. Niinomi M (1998) Mechanical properties of biomedical titanium alloys. Mater Sci Eng A 243:231–236. https://doi.org/10.1016/S0921-5093(97)00806-X

23. Shah FA, Trobos M, Thomsen P, Palmquist A (2016) Commercially pure titanium (cp-Ti) versus titanium alloy (Ti6Al4V) materials as bone anchored implants - Is one truly better than the other? Mater Sci Eng C 62:960–966. https://doi.org/10.1016/j.msec.2016.01.032

24. Kuromoto NK, Simão RA, Soares GA (2007) Titanium oxide films produced on commercially pure titanium by anodic oxidation with different voltages. Mater

Charact 58:114–121. https://doi.org/10.1016/j.matchar.2006.03.020

25. Lara Rodriguez L, Sundaram PA, Rosim-Fachini E, et al (2014) Plasma electrolytic oxidation coatings on γtiAl alloy for potential biomedical applications. J Biomed Mater Res - Part B Appl Biomater 102:988–1001. https://doi.org/10.1002/jbm.b.33079

26. Noumbissi S, Scarano A, Gupta S (2019) A Literature Review Study on Atomic Ions Dissolution of Titanium and Its Alloys in Implant Dentistry. Mater (Basel, Switzerland) 12:. https://doi.org/10.3390/ma12030368

27. Addison O, Davenport AJ, Newport RJ, et al (2012) Do “passive” medical titanium surfaces deteriorate in service in the absence of wear? J R Soc Interface 9:3161–4. https://doi.org/10.1098/rsif.2012.0438

28. Souza JCM, Barbosa SL, Ariza EA, et al (2015) How do titanium and Ti6Al4V corrode in fluoridated medium as found in the oral cavity? An in vitro study. Mater Sci Eng C 47:384–393. https://doi.org/10.1016/j.msec.2014.11.055

29. Nuevo-Ordóñez Y, Montes-Bayón M, Blanco-González E, et al (2011) Titanium release in serum of patients with different bone fixation implants and its interaction with serum biomolecules at physiological levels. Anal Bioanal Chem 401:2747–2754. https://doi.org/10.1007/s00216-011-5232-8

30. Ribeiro AR, Gemini-Piperni S, Travassos R, et al (2016) Trojan-Like Internalization of Anatase Titanium Dioxide Nanoparticles by Human Osteoblast Cells. Sci Rep 6:23615. https://doi.org/10.1038/srep23615

31. Baan R, Straif K, Grosse Y, et al (2006) Carcinogenicity of carbon black, titanium dioxide, and talc. Lancet Oncol 7:295–296. https://doi.org/10.1016/S1470-2045(06)70651-9

32. Boisen AMZ, Shipley T, Jackson P, et al (2012) NanoTIO2 (UV-Titan) does not induce

ESTR mutations in the germline of prenatally exposed female mice. Part Fibre Toxicol 9:. https://doi.org/10.1186/1743-8977-9-19

33. Girardello F, Custódio Leite C, Vianna Villela I, et al (2016) Titanium dioxide nanoparticles induce genotoxicity but not mutagenicity in golden mussel Limnoperna fortunei. Aquat Toxicol 170:223–228. https://doi.org/10.1016/j.aquatox.2015.11.030

34. Jain AK, Senapati VA, Singh D, et al (2017) Impact of anatase titanium dioxide nanoparticles on mutagenic and genotoxic response in Chinese hamster lung fibroblast cells (V-79): The role of cellular uptake. Food Chem Toxicol 105:127–139. https://doi.org/10.1016/j.fct.2017.04.005

35. Zdravković TP, Zdravković B, Lunder M, Ferk P (2019) The effect of micro-sized titanium dioxide on WM-266-4 metastatic melanoma cell line. Bosn J Basic Med Sci 19:60–66. https://doi.org/10.17305/BJBMS.2018.3674

36. Bhatavadekar NB (2012) Squamous cell carcinoma in association with dental implants: An assessment of previously hypothesized carcinogenic mechanisms and a case report. J. Oral Implantol. 38:792–798

37. Happe A, Sielker S, Hanisch M, Jung S (2019) The Biologic Effect of Particulate Titanium Contaminants of Dental Implants on Human Osteoblasts and Gingival Fibroblasts. Int J Oral Maxillofac Implants 34:673–680. https://doi.org/10.11607/jomi.6929

38. Yang F, Tang J, Dai K, Huang Y (2019) Metallic wear debris collected from patients induces apoptosis in rat primary osteoblasts via reactive oxygen species-mediated mitochondrial dysfunction and endoplasmic reticulum stress. Mol Med Rep 19:1629–1637. https://doi.org/10.3892/mmr.2019.9825

39. Vallès G, González-Melendi P, Saldaña L, et al (2008) Rutile and titanium particles

differentially affect the production of osteoblastic local factors. J Biomed Mater Res - Part A 84:324–336. https://doi.org/10.1002/jbm.a.31315

40. Rutkunas V, Bukelskiene V, Sabaliauskas V, et al (2015) Assessment of human gingival fibroblast interaction with dental implant abutment materials. J Mater Sci Mater Med 26:. https://doi.org/10.1007/s10856-015-5481-8

41. Suárez-López Del Amo F, Rudek I, Wagner VP, et al Titanium Activates the DNA Damage Response Pathway in Oral Epithelial Cells: A Pilot Study. Int J Oral Maxillofac Implants 32:1413–1420. https://doi.org/10.11607/jomi.6077

42. He X, Hartlieb E, Rothmund L, et al (2015) Intracellular uptake and toxicity of three different Titanium particles. Dent Mater 31:734–744. https://doi.org/10.1016/j.dental.2015.03.017

43. Wang ML, Tuli R, Manner PA, et al (2003) Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles. J Orthop Res 21:697–707. https://doi.org/10.1016/S0736-0266(02)00241-3

44. Kraft CN, Diedrich O, Burian B, et al (2003) Microvascular response of striated muscle to metal debris. A comparative in vivo study with titanium and stainless steel. J Bone Joint Surg Br 85:133–141. https://doi.org/10.1302/0301-620X.85B1.12749

45. Dodo CG, Meirelles L, Aviles-Reyes A, et al (2017) Pro-inflammatory analysis of macrophages in contact with titanium particles and porphyromonas gingivalis. Braz Dent J 28:428–434. https://doi.org/10.1590/0103-6440201701382

46. Pettersson M, Kelk P, Belibasakis GN, et al (2017) Titanium ions form particles that activate and execute interleukin-1$β$ release from lipopolysaccharide-primed macrophages. J Periodontal Res 52:21–32. https://doi.org/10.1111/jre.12364

47. Valles G, Gonzalez-Melendi P, Gonzalez-Carrasco JL, et al (2006) Differential

inflammatory macrophage response to rutile and titanium particles. Biomaterials 27:5199–5211. https://doi.org/10.1016/j.biomaterials.2006.05.045

48. Wheelis SE, Wilson TG, Valderrama P, Rodrigues DC (2018) Surface characterization of titanium implant healing abutments before and after placement. Clin Implant Dent Relat Res 20:180–190. https://doi.org/10.1111/cid.12566

49. Sato S, Kishida M, Ito K (2004) The Comparative Effect of Ultrasonic Scalers on Titanium Surfaces: An In Vitro Study. J Periodontol 75:1269–1273. https://doi.org/10.1902/jop.2004.75.9.1269

50. Khraisat A, Abu-hammad O, Al-kayed AM (2004) Stability of the Implant / Abutment Joint in a. Clin Implant Dent Relat Res 6:222–229

51. Zembic A, Kim S, Zwahlen M, Kelly JR (2014) Systematic Review of the Survival Rate and Incidence of Biologic, Technical, and Esthetic Complications of Single Implant Abutments Supporting Fixed Prostheses. Int J Oral Maxillofac Implants 29:99–116. https://doi.org/10.11607/jomi.2014suppl.g2.2

52. Schwarz MS (2000) Mechanical complications of dental implants. Clin Oral Implants Res 11 Suppl 1:156–158. https://doi.org/10.1034/j.1600-0501.2000.011S1156.x

53. Bolzoni L, Esteban PG, Ruiz-Navas EM, Gordo E (2012) Mechanical behaviour of pressed and sintered titanium alloys obtained from master alloy addition powders. J Mech Behav Biomed Mater 15:33–45. https://doi.org/10.1016/j.jmbbm.2012.05.019

54. Souza JCM, Henriques M, Oliveira R, et al (2010) Do oral biofilms influence the wear and corrosion behavior of titanium? Biofouling 26:471–8. https://doi.org/10.1080/08927011003767985

55. Manaranche C, Hornberger H (2007) A proposal for the classification of dental alloys according to their resistance to corrosion. Dent Mater 23:1428–1437.

https://doi.org/10.1016/j.dental.2006.11.030

56. Kirmanidou Y, Sidira M, Drosou ME, et al (2016) New Ti-Alloys and Surface Modifications to Improve the Mechanical Properties and the Biological Response to Orthopedic and Dental Implants: A Review. Biomed Res. Int. 2016

57. Flatebø RS, Johannessen AC, Grønningsæter AG, et al (2006) Host Response to Titanium Dental Implant Placement Evaluated in a Human Oral Model. J Periodontol 77:1201–1210. https://doi.org/10.1902/jop.2006.050406

58. Souza JCM, Ponthiaux P, Henriques M, et al (2013) Corrosion behaviour of titanium in the presence of Streptococcus mutans. J Dent 41:528–534. https://doi.org/10.1016/j.jdent.2013.03.008

59. Huang GY, Jiang HB, Cha JY, et al (2017) The effect of fluoride-containing oral rinses on the corrosion resistance of titanium alloy (Ti-6Al-4V). Korean J Orthod 47:306–312. https://doi.org/10.4041/kjod.2017.47.5.306

60. Souza JCM, Barbosa SL, Ariza E, et al (2012) Simultaneous degradation by corrosion and wear of titanium in artificial saliva containing fluorides. Wear 292–293:82–88. https://doi.org/10.1016/j.wear.2012.05.030

61. Catarina S (2016) Tribocorrosion and Bio-Tribocorrosion in the Oral Environment : The Case of Dental Implants Tribocorrosion and Bio- Tribocorrosion in the Oral Environment : The Case of Dental Implants

62. Messias A, Rocha S, Calha N, et al (2017) Effect of intentional abutment disconnection on the micro-movements of the implant–abutment assembly: a 3D digital image correlation analysis. Clin Oral Implants Res 28:9–16. https://doi.org/10.1111/clr.12607

63. Matusiewicz H (2014) Potential release of in vivo trace metals from metallic medical

implants in the human body: From ions to nanoparticles - A systematic analytical review. Acta Biomater 10:2379–2403. https://doi.org/10.1016/j.actbio.2014.02.027

64. Delgado-Ruiz R, Romanos G (2018) Potential Causes of Titanium Particle and Ion Release in Implant Dentistry: A Systematic Review. Int. J. Mol. Sci. 19

65. Flatebø RS, Høl PJ, Leknes KN, et al (2011) Mapping of titanium particles in peri-implant oral mucosa by Laser Ablation Inductively Coupled Plasma Mass Spectrometry and high-resolution optical darkfield microscopy. J Oral Pathol Med 40:412–420. https://doi.org/10.1111/j.1600-0714.2010.00958.x

66. Geurtsen W (2002) Biocompatibility of dental casting alloys. Crit. Rev. Oral Biol. Med. 13:71–84

67. Souza W, Piperni SG, Laviola P, et al (2019) The two faces of titanium dioxide nanoparticles bio-camouflage in 3D bone spheroids. Sci Rep 9:9309. https://doi.org/10.1038/s41598-019-45797-6

68. Takahashi K, Takashiba S, Nagai A, et al (1994) Assessment of Interleukin-6 in the Pathogenesis of Periodontal Disease. J Periodontol 65:147–153. https://doi.org/10.1902/jop.1994.65.2.147

69. Tawse-Smith A, Payne AGT, Kumara R, Thomson WM (2002) Early loading of unsplinted implants supporting mandibular overdentures using a one-stage operative procedure with two different implant systems: a 2-year report. Clin Implant Dent Relat Res 4:33–42

70. Kumazawa R, Watari F, Takashi N, et al (2002) Effects of Ti ions and particles on neutrophil function and morphology. Biomaterials 23:3757–3764. https://doi.org/10.1016/S0142-9612(02)00115-1

71. Shanbhag AS, Jacobs JJ, Black J, et al (1994) Macrophage/particle interactions: Effect

of size, composition and surface area. J Biomed Mater Res 28:81–90. https://doi.org/10.1002/jbm.820280111

72. Zhang Y, Yu W, Jiang X, et al (2011) Analysis of the cytotoxicity of differentially sized titanium dioxide nanoparticles in murine MC3T3-E1 preosteoblasts. J Mater Sci Mater Med 22:1933–1945. https://doi.org/10.1007/s10856-011-4375-7

73. Sund J, Palomäki J, Ahonen N, et al (2014) Phagocytosis of nano-sized titanium dioxide triggers changes in protein acetylation. J Proteomics 108:469–483. https://doi.org/10.1016/j.jprot.2014.06.011

74. Souza PPC, Lerner UH (2013) The role of cytokines in inflammatory bone loss. Immunol Invest 42:555–622. https://doi.org/10.3109/08820139.2013.822766

75. Darowish M, Rahman R, Li P, et al (2009) Reduction of particle-induced osteolysis by interleukin-6 involves anti-inflammatory effect and inhibition of early osteoclast precursor differentiation. Bone 45:661–668. https://doi.org/10.1016/j.bone.2009.06.004

76. Steeve KT, Marc P, Sandrine T, et al (2004) IL-6, RANKL, TNF-alpha/IL-1: Interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 15:49–60. https://doi.org/10.1016/j.cytogfr.2003.10.005

77. Goodman SB, Ma T, Chiu R, et al (2006) Effects of orthopaedic wear particles on osteoprogenitor cells. Biomaterials 27:6096–6101. https://doi.org/10.1016/j.biomaterials.2006.08.023

78. Gill SE, Parks WC (2008) Metalloproteinases and their inhibitors: Regulators of wound healing. Int J Biochem Cell Biol 40:1334–1347. https://doi.org/10.1016/j.biocel.2007.10.024

79. Zhang X, Morham SG, Langenbach R, et al (2001) Evidence for a direct role of cyclo-

oxygenase 2 in implant wear debris-induced osteolysis. J Bone Miner Res 16:660–670. https://doi.org/10.1359/jbmr.2001.16.4.660

80. Perry MJ, Ponsford FM, Mortuza FY, et al (1996) Osteolytic properties of the synovial-like tissue from aseptically failed joint prostheses. Br J Rheumatol 35:943–950. https://doi.org/10.1093/rheumatology/35.10.943

81. Theoleyre S, Wittrant Y, Tat SK, et al (2004) The molecular triad OPG/RANK/RANKL: Involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev 15:457–475. https://doi.org/10.1016/j.cytogfr.2004.06.004