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Innovative surfaces and alloys for dental implants: What about biointerface-safety concerns?

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ABSTRACT

Objectives. The present review article aimed to discuss the recent technologies employed for the development of dental implants, mainly regarding innovative surface treatments and alternative alloys, emphasizing the bio-tribocorrosion processes.

Methods. An electronic search applying specific MeSH terms was carried out in PubMed and Google Scholar databases to collect data until August 2021, considering basic, pre-clinical, clinical and review studies. The relevant articles (n = 111), focused on innovative surface treatments for dental implants and their potential undesirable biological effects, were selected and explored.

Results. Novel texturization methodologies for dental implants clearly provided superficial and structural atomic alterations in micro- and nanoscale, promoting different mechanical-chemical interactions when applied in the clinical set. Some particulate metals released from implant surfaces, their degradation products and/or contaminants exhibited local and systemic reactions after implant installation and osseointegration, contributing to unexpected treatment drawbacks and adverse effects. Therefore, there is an urgent need for development of pre-clinical and clinical platforms for screening dental implant devices, to predict the biointerface reactions as early as possible during the development phases.

Significance. Modern surface treatments and innovative alloys developed for dental implants are not completely understood regarding their integrity during long-term clinical function, especially when considering the bio-tribocorrosion process. From this review, it is possible to assume that degradation and contamination of dental surfaces might be associated within peri-implant inflammation and cumulative long-lasting systemic toxicity. The in-depth comprehension of the biointerface modifications on these novel surface treatments might preclude unnecessary expenses and postoperative complications involving osseointegration failures.

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Contents

1. Introduction

In recent years, there has been a huge range of surface treatments proposed to modify and accelerate the osseointegration processes [1–3]. Different methodologies are employed aiming to alter morphology, roughness, crystalline phase and wettability, without impairing the biocompatibility [1–5]. Therewith, processes using particles for blasting, acid solutions, heat treatments, among others, are applied to the implants, often involving chemical elements different from the chemical composition of the implant-base material, to modify the atomic formatting of the surface for better performance toward cell adhesion [1–3,6–9].

Blasting with aluminum oxide (Al_2O_3), titanium (Ti) and silica (SiO_2) particles, acid etches involving sulfuric acid/hydrochloric acid, or combinations of these methods are commonly used on implant surfaces [1,2,7–9]. However, these materials or solutions can interact intrinsically or superficially with the biomedical implant surfaces, requiring subsequent cleaning steps. Research reports revealed the presence of particles of chemical elements such as aluminum (Al), under microscopic analysis, in Ti commercially available implants [7,10,11]. In addition, Beger et al. reported findings showing the presence of Al on the surface of zirconia-based (ZrO_2) commercial implants, by means of energy-dispersive X-ray spectroscopy (EDX) [12]. As a conclusion, these investigations suggest some impregnation or impurity accumulation during the manufacturing of the implant. On the other hand, modern surface treatments might achieve topographies with micro- and nano-scale that promote huge improvement in biocompatibility with peri-implant tissues [2,4,5,13]. Currently, surfaces with nanostructured morphology allied to hydrophilic characteristics demonstrated the best tissue responses around modified surfaces for dental implants [13].

Simultaneously, the release of micro or nanoparticles of chemical elements from medical devices has been the focus of current investigations, due to the advances of analytical technologies [14–16]. Some elements can generate cellular toxicity; others can be transported and eliminated by the body or be stored in some tissues [14,17–19]. The long-term behavior of fragments of certain chemical elements in tissues or cells, at the molecular level, is still not well understood, requiring further studies. Thus, there is a current need for in-depth basic and clinical studies to monitor the potential safety problems regarding those implants. Furthermore, surfaces for dental/biomedical implants with drug delivery systems have been proposed based on pre-clinical studies, for the release of metal nanoparticles, such as gold, silver and copper, aiming to inhibit the infectious agents [5,20]. These strategies require additional investigation concerning the possible toxicity of these substances, especially in a long-term basis.

Therewith, this critical review article summarized the main methodologies applied to surfaces of commercial Ti, ZrO_2 and Ti-based alloy implants, explaining their processes and reporting alterations that may influence their surface composition. Special attempts have been made to cover the possible cytotoxic and inflammatory effects of chemical elements added to or existent in the implant surfaces (intra-osseous and extra-osseous) after contact and function with living tissues.

2. Search strategy

An electronic search in PubMed and Google Scholar databases was performed to identify *in vitro*, *in vivo* and clinical studies published until August 2021, with focus on innovative surface treatments for dental implants and the possible negative biological impacts. The electronic search was carried out using the following keywords and MeSH terms: "implants" or "dental implants" and "corrosion" or "biocorrosion" or "biotribology" or "tribology" or "impurities" or "contamination" or "alloys" or "metal release" and "cells" or "bone cells" or "osteoblasts" or "mesenchymal cells" or "biological response" or "cellular response" or "fibroblasts" or "epithelial cells" or "macrophages" or "immune response" or "cytotoxicity". The inclusion criteria considered for this critical review was: (1) English-written studies, (2) systematic reviews, (3) critical reviews, (4) clinical trials, (5) animal, and (6) *in vitro* studies. Two reviewers (M.F.K. and T.C.M.) evaluated individually the content of possible relevant studies for this review. The main studies (n = 111) were then selected independently and analyzed to summarize and discuss the surface treatment methods, dental implant surface alterations, the bio-tribocorrosion process and the possible negative reactions related with the different altered-surfaces and alloys.

3. Main surface treatments applied for commercial dental implants

3.1. *Machining*

The first process to be applied in the manufacture of biomedical/dental implants based on Branermark's studies [21] was machining. This process involves large lathes for the implant designs, drawing them with details using harder metals for deformation of the base material, together with high rotation speed. The surface characteristics obtained with this process maintain a roughness on macro- or micro-scale, requiring a longer period for bone healing as 4–6 months for rehabilitation [21,22]. Currently, some lathes are already digitally controlled, accelerating the process of confection and decreasing the chance of human errors in the process [23].

3.2. *Grit blasting*

Considering the need for a progress in the level of roughness of implant surfaces for osseointegration in poorer bone sites, processes such as blasting of micro-, or nanoparticles have emerged due to their feasibility and low costs. The process involves the application of particles, usually Al_2O_3, Al, Ti, or hydroxyapatite, in high-pressure and high-speed sandblaster to bombard the implant surface [1,2,7,9,12]. Therewith, countless depressions are created by the deformation of the base material used, and the size of these deformations depends on the composition of the applied particle as well as its size. Roughness on a micro- or nanoscale can be achieved, usually without standardization when measuring the entire surface [1,2]. The implementation of this process improved the osseointegration time, when compared with machined or smooth surfaces [1,2,6,22].

3.3. *Acid etching*

The application of acid solutions is of great value in many areas of biotechnology for developing roughness or cleaning surfaces. Its use might widely vary according to the composition, concentration, temperature and time of the process. Thereby, a range of different topographies can be developed with its use [1,2,7,8]. As it involves a chemical reaction on the implant surfaces, many residues remaining from the implant's manufacturing are eliminated or destroyed. As for surface morphology, numerous topographic formations are reported [1,2,24,25], determined by the variation of methodologies in the application of acid attacks. Roughness and varied morphologies on both micro- and nanoscales have been reported [24,25], and the utilization evolved regarding osseointegration speed and morphology, with a decrease in biofilm formation [24,25]. However, its exaggerated application can create high wrinkles and superficial deformations, which are reported as not beneficial for cell healing [26]. Thus, methodology standardization and inspection of processes must be rigorous to create an ideal topography on implant surfaces.

3.4. *Sandblasting plus acid etching*

Currently, the most employed surface treatment process by commercial implant companies is the combination of blasting with acid etching, also known as s̈and-blasted large grit acid etched[̈1,2]. The topography developed by the combination of these processes proved to accelerate both osseointegration and cell adhesion, reaching osseointegration success within 1–2 months [1,2,6,8,27,28]. The process allows for greater variation in surface morphology as it involves deformations by physical contact (blasting) and irregularities by chemical action (acid etching) [1,2,6,8]. In addition, the process combination is s̈elf-helpd̈ue to the possibility of acidic agents cleaning any remaining impurities from blasting. It is considered the most effective surface treatment process in the literature, according to pre-clinical and clinical studies, showing elevated long-term success rates [8,27,28]. However, the blasting-acid etched combined approach applied by different companies are varied and commonly present intellectual property, making it difficult to critically evaluate this process and what would be the correct parameters to be applied for an ideal surface with the desired morphology, roughness and cleanliness.

3.5. *Anodizing*

In recent years, an electrochemical process has gained attention for its performance in a morphological form (nanotubes/nanoporous) on surfaces, and industries have been using this technology in biomedical implants [5,20,24,29]. Surface anodizing might involve an electrolyte solution, changes in temperature and voltage, as well as the use of a cathode and an anode surface to complete the process [5,20,24]. This treatment can also be combined with acid etching, blasting or machined surfaces. Meanwhile, most studies present combi-

Table 1 – Main surface treatments employed for commercial implants.

Surface Treatment	Based material applied	Process/equipment and compounds employed	Surface topography scale	References
Machining	Ti and Ti alloys	Mechanical lathe	Macro/micro	[2,21,24]
Grit blasting	Ti, ZrO_2, ceramics and Ti alloys	Particle blasting with sand, alumina, Al, Ti, Al_2O_3, silica, hydroxyapatite	Micro/nano	[7,9–12]
Acid etching	Ti, ZrO_2, ceramics and Ti alloys	Acid solutions with different concentrations, temperature, and time of application. Commonly, sulphuric and chloridric acids are used	Micro/nano	[2,24,34]
Grit blasting plus acid etching	Ti, ZrO_2, ceramics and Ti alloys	Different combinations of grit blasting and acid etching	Micro/nano	[7,8,10,27,28]
Anodizing	Ti, ZrO_2 and Ti alloys	Electrochemical equipment involving different electrochemical solutions, temperatures, times, and voltage	Micro/nano	[20,30,31,35]
Plasma-spraying	Ti, ZrO_2 and Ti alloys	Creation of thin films over the surface using a plasma torch under vacuum. Different materials may be applied such as Ti, Au, Ag and ceramics.	Micro/nano	[3,33]

nation with acid etches [24] or smooth surfaces for the growth of oriented nanotubes [30]. Its presentation on a nanomorphological scale reveals better results for osseointegration in periods of one to two months [2,31]. Furthermore, intense scientific research is currently presented about the functionalization of this type of surface for drug delivery systems [5,20].

3.6. *Plasma-spraying*

The concept of biomimeticing implant surfaces with bone characteristics emerged with the application of hydroxyapatite/calcium coatings, which could provide a contact surface with an atomic composition similar to the elemental composition of bone [32,33]. The coating is applied to the implant surface using a plasma system loaded with the desired material in a vacuum or low atmospheric pressure environment. Thus, the new deposited layer adheres and is formed by melting and sintering [2]. Its advantages demonstrated to be the potential for creating surface layers in micro- and nanoscale, as well as the application of different materials for the development of these coatings such as titanium, gold, silver and several ceramics [2]. Disadvantages revealed the need for extreme surgical care in the clinical insertion of implants with this superficial treatment because its interfacial fragility (implant-coating) is greater, and fracture of the adhered layer might occur, in addition to a propensity for greater bacterial contamination of this type of superficial treatment [32].

The most common surface treatments found in the implant market are summarized in Table 1. In addition, examples of the above-mentioned processes are shown in Fig. 1.

4. Innovative processes for dental implant surfaces under research stage for commercial application

4.1. *Laser treatment*

In order to maintain a textured pattern with well-ordered peaks and valleys at the micro- or nanoscale, some researchers described the use of laser-induced surface treatments [36–38]. With the application of laser processing, identical and constant morphologies could be manufactured, providing better cell adhesion and proliferation along surfaces [36]. Zwarh et al. showed the confection of complex morphologies on Ti surfaces with the application of direct laser interference patterning (DLIP), thus demonstrating a 16% increase in the viability of osteogenic cells, when compared with standard surface treatments (sand-blasted/acid-etched) [37]. They have also demonstrated the successful application of laser treatment for commercial dental implants [37]. Additionally, the surface treatment process with lasers proved to be efficient in all base materials applied to dental implants, such as Ticp, Ti alloys and several ceramics [38].

4.2. *Alkali-based treatment*

Alkali treatments are based on exposing the implant surface in solutions normally composed of NaOH or CaP, which can be submitted to a heating treatment or not. From this exposure, a thin micro- or nanoscale layer (alkali-titanate layer) is formed, modifying the base material interface [2]. Camargo et al. revealed similar results when they evaluated the insertion of implants in Wistar rats with alkali-treated, sand-blast treated and acid-etch treated surfaces, showing that the quality of osseointegration with this treatment is equivalent to established treatments [39]. When a methodology including a hydrothermal treatment under alkali conditions was applied, a surface nanotopography with hydrophilicity characteristics has been achieved [40]. Thus, authors revealed favorable conditions for cell proliferation and bone adhesion in an *in vivo* model after twelve weeks of osseointegration [40]. However, the optimization of protocols for this surface treatment is still not completely clear and there is a need for further studies for a standardization procedures.

4.3. *Bioprinting*

With the evolution of digital technologies for dentistry, some researchers have proposed the development of dental

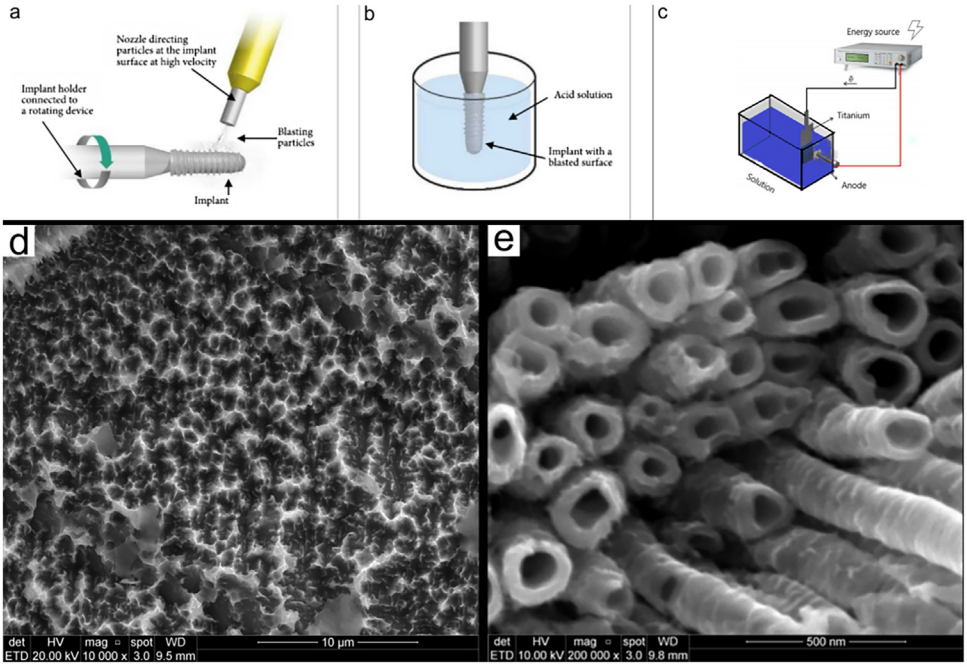

Fig. 1 – Schematic examples of surface treatments: blasting (a); acid etching (b); anodizing (c) and scanning electron microscopy from acquired topography using double acid etching (d) and anodizing (e). Reproduced and adapted with permission under the terms of the Creative Commons Attribution 4.0 International license (CC BY 4.0) – a and b from reference [7]; c and e from reference [20].

implants by 3D-fabrication [23]. These implants are usually manufactured in Ti, Ti alloys or ZrO_2 by additive or subtractive manufacturing processes [23]. A great advantage is the potential of customizing the implant for each clinical case and the digitalization of the implant manufacturing process. However, the surface quality demonstrated by most processes used for 3D-fabrication of dental implants still proves to be inefficient for fabricating perfect surfaces in micro- and nanoscale. Thus, post-3D confection basic surface treatments are required to improve the implant surface quality, reducing one of the great advantages of this manufacturing model, which would be the distance from industrial processes [23]. It is proposed that certain technological evolution is still needed to safely reach commercial standards.

4.4. *Biodegradable coatings*

With the aim to improve some surface properties, such as greater osteogenic cell adhesion, and to decrease bacterial proliferation, techniques employing the addition of biodegradable materials on the surfaces of dental implants have been proposed. Coatings composed of different molecules show interesting results with implantable surfaces such as polysaccharides, chitosan, peptides, collagen, degradable polymers, among others [41]. However, the application of coatings on surfaces that had been already treated with characteristics of hydrophilicity and/or nanostructuring might impair some of these properties, requiring a deep investigation to find out if their increase provides or not additional advantages. Kazek-Kesik et al. demonstrated the development of a coating composed of a polymer (PLGA) loaded with amoxicillin on Ti surfaces for dental implants that resulted in significant improvements in both biocompatibility and antibacterial properties, even with a decrease in surface hydrophilicity [42]. Even so, a large part of these new technologies is only in the preliminary research phase and cannot yet be found in the dental market.

5. Surface quality: Effects derived due surface treatments

5.1. *Morphology, roughness and hydrophilicity*

Deformations, roughness, chemical ruptures, crystallinity alterations, and oxide layers are characteristic of surfaces targeted by the processes mentioned above. Modifications in the level of crystalline structure and morphology are considered essential for a better interaction and cell adhesion [2,4]. Thereby, a spreading process and greater cellular intercommunication, ending in a stage of tissue neoformation around the surface is expected [4,34,43]. The different degrees of roughness, morphology, or free surface energy reveal different cell reactions in the first contact stage [26,44]. Dissimilar surface treatments have different waiting times for osseointegration. All models of surface treatments are expected to favor the speed of osseointegration compared with untreated surfaces [1–3,34,44]. It has been suggested that cells interact better with the surface in an approximate roughness average of 1.5 µm [26] and their initial communication is supported by nanoscale surfaces, such as nanotubes or nanoporous, providing similar scaled interactions with extracellular structures [24,29].

Additionally, the degree of wettability of a surface designated for a dental implant directly influences the early cellular response of osseointegration. The set of physical modifications (morphology/roughness) combined with chemical modifications (hydrophilicity) presents the most interesting responses for osseointegration [13]. Usually, most of the surface treatments mentioned in previous chapters provide the surface treated with hydrophobic characteristics. However, a second stage of subsequent surface treatments has been applied in order to change these characteristics to surfaces called superhydrophilic [5,13]. Processes such as UV-light applications, use of plasmas, conservation of the implant in specific solutions, polyelectrolytic modifications, among others, have been reported as important methods to achieve superhydrophilic properties and greater healing speed [13]. In the current implant market, liquid solutions are most used with the implant capsule to maintain the high degree of hydrophilicity until its application to the patient, extending its rapid osseointegration properties until the removal of its storage capsule [45].

It is tempting to propose that progress in the last 50 years with different surface treatments has been essential for the quality and speed of clinical treatment to achieve excellence in rehabilitation procedures. However, a critical quality control is necessary in all parts of the manufacturing process and in the acquisition of different base materials for biomedical implants.

5.2. *Chemical surface composition and impurities*

Grade IV titanium and Ti6Al4V alloy are the base materials most found in Ti implants, due to their strength, biocompatibility and excellent osseointegration results [1,2,10,46]. In addition, implants derived from ZrO_2 are widely employed due to the interest in their more äestheticëxtrinsic characteristics, besides the high biocompatibility [12,34,47]. Naturally, the atomic composition of the implant surfaces based on these materials must be equal to their internal composition. Meanwhile, some current findings show variations in atomic composition on the dental implant surface [7,10–12,48], as analyzed by scanning electron microscopy (SEM) and X-ray dispersive spectroscopy (EDX). For instance, Beger et al. (2018) detected small residues of Al on the surface of commercial dental implants derived from ZrO_2, suggesting some deficiency in the blasting and/or cleaning processes [12]. Furthermore, Guo et al. (2019) showed remnants of silica and alumina after blasting dental implant surfaces with these materials, suggesting an impregnation on the surface [48]. Additional reports revealed the presence of Al and Ni on the surface of a commercial dental implant, even after acid etching, according to the evaluation of atomic composition by SEM and EDX [10,11,49]. In addition, investigations have found Al fragments up to 5900-μm^2 in size (Fig. 2a) and a significant number of smaller particles on the entire surface of the implant where blasting plus acid etching was used [7]. Moreover, critical analyses with inductively coupled plasma-mass spectrometry and optical emission spectrometry revealed contamination of the base material of commercial zirconium implants [50]. Elements such as Al, Ni, Cr and traces of U-238 and Th-232 radionuclide contamination were also found in several samples [50]. However, most of the contaminants are within the ISO manufacturing standard values and further studies are needed to investigate the long-term toxic effects of these contaminants.

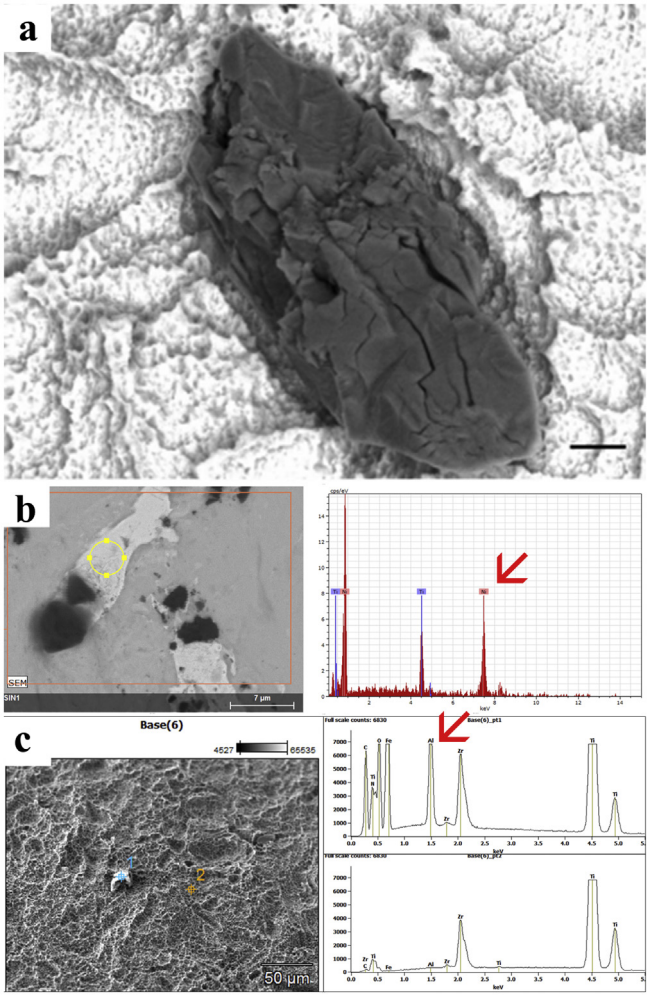

Fig. 2 – Examples of unexpected impurities on the commercial dental implant surfaces. Al particle encrusted on titanium implant (a); EDX spectrum from a commercial implant surface revealing nickel and titanium peaks (b); EDX spectrum and microscopy revealing Al impurities on the commercial implant surface (c). Red arrows shows the unexpected peaks. Reproduced and adapted with permission under the terms of the Creative Commons Attribution 4.0 International license (CC BY 4.0) – a from reference [7]; b from reference [10].

Detection of unexpected atomic elements in the commercial phase is indicative of some deficiency during the process of material purchase, treatment, cleaning and/or storage. Many of these chemical elements have different properties in relation to the base material. Noteworthy, in many cases, they have cytotoxic characteristics [15,17,18]. Particulate or nanoparticulate materials have a higher potential of interacting with cells, being actively transported or even degraded [19]. Even so, some metal fragments have properties that preclude degradation, which can compromise the healing processes.

6. Bio-tribocorrosion

After the insertion of an implantable metallic device, the human body provides an extremely complex and aggressive environment in terms of stability of physicochemical properties. Challenging conditions from wear and degradation processes through mechanical, chemical, biochemical and microbiological processes are inherent [51–56]. Thus, situations of friction, lubrication, and wear between interfaces, added to an environment with metabolic, immunological, chemical and microbiological processes, define and justify the bio-tribocorrosion of dental implants [51–56].

From a clinical perspective, the starting point of the bio-tribocorrosion phenomenon is the insertion of the dental implant. Frictional forces, torque and loading might cause damage or material loss at the surface/bone interface [52]. After that, the subsequent application of loads, intense and repetitive movements generate micro- or nano-movements in the implant, which can induce superficial rupture and release of particles and ions from the implant interface [52,53]. In addition, the saliva in the oral environment might also represent a complicating issue in this process. Normally, saliva has a stable pH (between 6 to 7); however, some factors such as feeding, hygiene and microbiota can reduce the pH, stimulating degradation due to the loss of resistance to corrosion [54]. On the other hand, studies revealed a reduction in friction and wear in metal implants that are in contact with saliva due to their lubrication and viscosity properties [55].

Metabolites generated by inflamed and/or infected environment can generate an intense tendency to biocorrosion [51,54,55]. Dead cells, as well as contaminated and inflamed regions have lower pH and oxygen vacancy leaving more reactive areas promoting corrosion [51,56]. The continuity of these processes over time causes surface failures and the release of particles/ions from the implant. Furthermore, a study with five different mini-implant systems, composed of Ti and Ti6Al4V alloys, revealed a significant release of Ti, Al, and V ions after 30 days of immersion in artificial saliva, showing that degradation of these alloys in induced oral environment does not depend on long periods [57].

According to the surface treatment methodology or the application environment of the implant, the phenomenon of bio-tribocorrosion might be intensified. Surface treatments are known to induce ruptures and changes in atomic bonds to create roughness and reactive layers aiming at greater cell adhesion [1,3,4,24]. However, these same treatments are associated with greater variables for corrosion, wear, friction and ruptures, which may induce the release of particles and ions to the adjacent tissues, compromising the device adaptation due to cytotoxic and inflammatory actions.

7. Cytotoxic and inflammatory influence of implant-containing elements

The application of different chemical elements and alloys in biomedical implants is extremely broad. A recent publication by the US Food and Drug Administration (FDA) regarding biological responses of metal implants used for different anatomical sites [58] revealed an extensive combination of metals and metal alloys in implant or orthodontic devices, such as gold, silver, palladium and alloys (mainly cobalt–chromium or nickel–chromium) [58,59]. For intraosseous implants (including dental implants), combinations with aluminum and vanadium are very common, in addition to new alloys from titanium, including tantalum, niobium, among other elements, pursuing greater quality in physical and chemical properties [58–60].

In an environment with biological activity, such as the mouth, metals behave differently than in a stable environment. Many metals or particles have a tendency for binding with free radicals –OH, enzymes and proteins, as well as in cell membranes with the correct polarization [61]. As a result, different changes can occur in the normal mechanisms of cells and tissues (*e.g.*, cell membrane polarization, changes in permeability, inflammatory reactions and decreased cellular respiration) [62]. Furthermore, metal ions or nanoparticulates promptly bind to proteins present in the blood through SH-group bonds [61]. Hence, these elements can be transported to various tissues or organs of the body, what might account to some grade of systemic toxicity.

A systematic review on aluminum effects showed that high levels of Al^{+3} can induce cytotoxicity by oxidative damage, depending on the solubility and dose [63]. Toxicity has been associated with the competition between Al and Fe ions along with the mitochondrial processes. As a result, there is a dysregulation in the cellular metabolic systems, impairing the cell survival [61]. Similarly, Kermani et al. described that Al_2O_3 nanoparticles can bind to Tau protein in SH-SY5Y neuroblastoma cells, promoting structural damage and cell death by apoptosis and necrosis [64]. A retrospective study conducted by Yuichi et al. demonstrated the possible migration of Al and V ions from spinal implants composed by the TiAl6V4 alloy. In their findings, a third of the patients (46 studied patients) had abnormal hair or serum metal concentrations, after an average of 5.1 years post-surgery, indicating that Ti and Al particles can travel throughout the human body after dissolving of metals [65] (Fig. 3).

In an *in vitro* study, the authors evaluated the viability of human fibroblasts on the surface of pure Ti and TiAl6V4, after 72 h of cell culture. Data showed significant differences for the alloy composed of Al and V, suggesting the influence of these elements on cell survival, without compromising the biocompatibility [66]. Similarly, Costa et al. demonstrated the release of ions and V particles from the Ti-6Al-V4 alloy and detected cytotoxic effects for this blend. The authors demonstrated a reduction of cell viability and significant differences in cell morphology, for MC3T3-E1 pre-osteoblasts and NIH3T3 fibroblasts, after exposure to high concentrations of V_2O_5. In addition, the same publication revealed the presence of this chemical element in the synovial fluid of patients with oral implants in function [67]. Nevertheless, the concentration found was considered low, but its effects should be evaluated in a long-term basis, to verify the potential toxicity for other organs. Alternatively, an *in vitro* evaluation reported the use of alloys such as Ti Nb-13Zr-13 and Ti Nb-35-Zr-7-Ta5 with no significant differences regarding the immediate cytotoxicity in relation to simpler alloys [60].

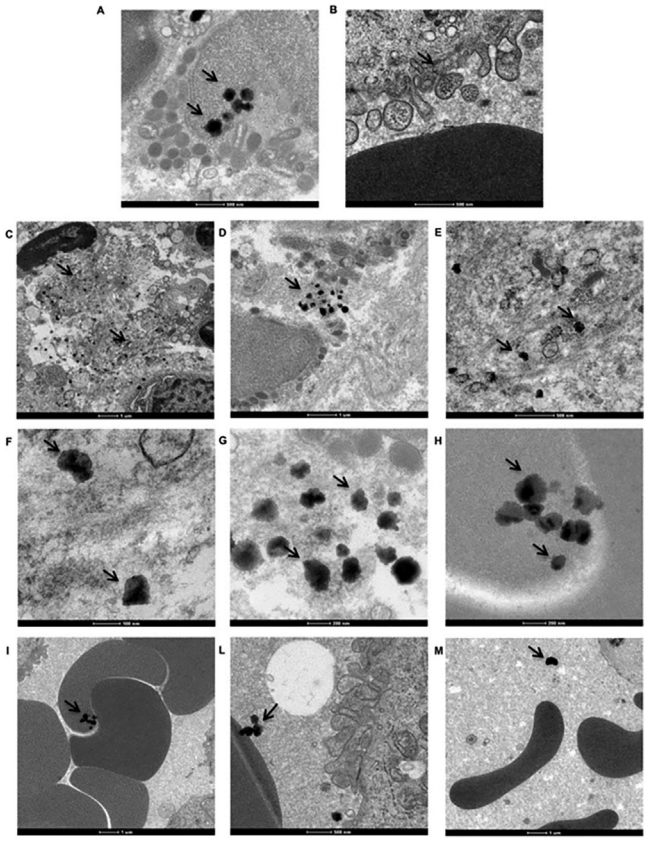

Fig. 3 – Electron microscopy revealing Ti particles (black arrows) in peri-implant tissues (A); inside cells (B); inside cells *via* endocytic vesicles (C–E); in the extracellular matrix (F–H) and associated with blood plasma (I–M). Reproduced with permission under the terms of the Creative Commons Attribution 4.0 International license (CC BY 4.0) from reference [14].

Isolated Ti particles also exhibited cytotoxic effects. In this regard, Bressan et al. [14] revealed a decrease in cell viability of undifferentiated cells and fibroblasts, when in contact with Ti nanoparticles. Moreover, histological findings showed a degradation of collagen fibers and changes in blood vessels, interfering with bone healing rates [14]. Additional evidence suggested that Ti particles are degraded from the implant surfaces over time and exposed to peri-implant tissues [14,15,68]. It has been proposed that minimal degradation levels display marked effects on inflammatory signaling pathways, greatly impacting the cellular behavior near to the implants, which might account for some grade of systemic toxicity. Moreover, clinical evidence has been shown by Daubert et al. in the analysis of 15 dental implants in function for 10 years. Significant levels of titanium were found in submucosal plaque collections in peri-implant tissues from most patients considered to be ill. Accordingly, the authors concluded that the dissolution of Ti contributes to the modification of the peri-implantitis microbiota [69].

As previously reported [48], SiO_2 particles can be detected on dental implant surfaces, as this material has been currently used in several areas of biomaterial technology [70]. Marquardt et al. demonstrated a dose-dependent cytotoxicity for this agent, by evoking membrane disruption and death of the murine macrophages RAW 264.7. They also revealed that low concentrations of SiO_2 nanoparticles are sufficient to activate autophagy [70]. In addition, aluminum oxide (Al_3O_2) and silicon oxide (SiO_2) nanoparticles caused DNA damage in RAW 264.7 cells, an effect that was associated with the chemical changes (low pH) generated in cell vesicles for both tested materials [71].

Immunogenic metals can produce reactivity *in vitro*. M1 pro-inflammatory macrophages and lymphocytes are usually found in areas with chronic inflammation or repair processes. Fretwurst et al. detected the presence of elements such as Ti and iron (Fe) in soft and hard peri-implant tissues in nine of the 12 patients investigated. The presence of these chemical elements was associated with a greater presence of macrophages and lymphocytes according to histological analysis [72]. Additional reports showed that incubation of primary human lymphocytes with cobalt-chromium-molybdenum (Co–Cr–Mo) or titanium alloys (Ti-6Al-4V) stimulated lymphocyte activation, with an increase of cell proliferation. Degradation products from Cr and Ti alloys can display cytotoxic potential, leading to unwanted peri-implant and systemic effects [73].

Noteworthy, metal elements from biomedical implants can release bone-associated cytokines leading to osteolysis in patients with implants under function. Wang et al. [74] assessed the effects of soluble metals, namely titanium, cobalt and chromium, on the release of bone-associated cytokines in human blood monocytes/macrophages and monocyte-like U937 cells, under stimulation with bacterial lipopolysaccharide (LPS). They demonstrated that Ti was mostly associated with the release of pro-inflammatory cytokines IL-1β, IL-6, TNF. Conversely, the release of the anti-inflammatory cytokine TGF-β1 was inhibited by all the tested metals. Remarkably, Ti was also able to potentiate LPS-induced U937 cell proliferation. Aside, in this study, none of the assessed metals elicited cytotoxic effects.

Cal et al. investigated seven different types of dental implants presenting ultrastructural compositions such as Ti grade IV, Ti grade V, TiZr alloy and different surface treatments. Significant differences were found regarding cell viability and apoptosis, according to the evaluation of an osteoblastic cell line. The authors proposed that systems with greater purity and less variation in chemical composition should be chosen by clinicians for obtaining a superior biocompatibility [75].

Nickel–chromium alloys have been associated with cytotoxicity, due to mutagenic effects. Human gingival fibroblasts were analyzed by SEM after 72 h of incubation with salt solutions of beryllium (Be^{+2}), chromium (Cr^{+6} and Cr^{+3}), nickel (Ni^{+2}) or molybdenum (Mo^{+6}) ions. Many morphological, biochemical and histological alterations have been observed, such as nuclear deformation by Cr^{+6} and Ni, reduction of dilatation of rough endoplasmic reticulum and mitochondria by Be and Ni and decrease of polyribosomes and mitochondrial size by all of the tested ions. These data are suggestive of cell aging/death induction, metabolic injury, and possible carcinogenic/mutagenic effects for the tested alloys, making evident the need to develop new strategies to produce safer biomedical implant components [76]. Non-cytotoxic and

Table 2 – Biological responses to metals ions or particulates.

Metal or alloy	Type of study	Action in cells	Possible clinical implications	References
Vanadium ions	*In vitro*	Significant decrease in the fibroblasts cell viability with vanadium concentration (23 μM)	Suggested a possible contribution to peri-implantitis and the transport of this element by the body.	Costa et al. (2019) [67]
Ti particles	*In vitro and in vivo*	Ti particles induce ROS production in human stem cells.	*In vivo* findings suggest that the release of Ti particles causes a possible gene deletion, and consequently a deregulation of the bone regeneration process.	Bressan et al. (2019) [14]
Ti ions	*In vitro*	Ti ions form particles and induced IL-1b release from human macrophages.	Suggested a secondary stimulus for peri-implant disease.	Pettersson et al. (2016) [80]
Ti-6Al-4V alloy	*In vitro*	Ions released from alloy can cause DNA and nuclear damage hamster ovary cells.	Suggested careful analysis of the potential cytotoxicity by metal alloys in medical implants.	Gomes et al. (2011) [81]
Al_3O_2 and SiO_2 nanoparticles	*In vitro*	Nanoparticles concentration high 200 μg/mL caused cytotoxic and genotoxic to macrophages.	Suggested careful in the use of nanoparticles in the oral environment.	Hashimoto et al. (2015) [71]
Al_3O_2 and TiO_2 nanoparticles	*In vitro*	Nanoparticles caused viability decrease in UMR 106 cells after 96 h of culture.	Suggested that events caused by cytotoxic effects can induce osseointegration failures.	Virgilio et al. (2010) [82]
Ti-6Al-7Nb alloy	*In vitro and in vivo*	Decreased viability in Saos-2 cells compared to another Ti alloy.	*In vivo* findings presented osseointegration, however, blood analysis showed hematocrit lower than average.	El-Hadad et al. (2018) [83]
Silica microparticles	*In vitro*	Induced apoptosis of human periosteal cells and decrease in proliferation and viability.	Acute cytotoxic effect where silica microparticle can be implanted.	Masuki et al. (2020) [84]
Silicon carbide nanoparticles (nanowires/control)	*In vitro*	Silicon carbide nanowires are toxic to human MSCs (0.1 mg/mL).	Possible toxic in the application situ.	Chen et al. (2018) [85]
Vanadium ions	*In vitro*	Significant decrease in cellular viability of human monocytes with concentrations above 3 μM. Also, viability reduction in MSCs cells.	–	Konig et al. (2017) [86] Zhang et al. (2018) [87]

low-cost elements, such as manganese and molybdenum, are interesting candidates for the development of alloys for biomedical implants. The measurement of indirect cytotoxicity after 48-h exposure of mouse L929 fibroblastic cells to different Ti–Mo–Mn alloy extracts showed low cytotoxicity, with no changes of cell morphology [77]. The main effects of chemical elements present in biomedical implants technologies (alloys or surface treatment processes) are summarized in Table 2.

Ions or particles released from the surfaces of biomedical implants are not fully biocompatible; residues derived from the bio-tribocorrosion process might display an immunogenic potential leading to a series of inflammatory changes [88]. Pettersson et al. revealed that isolated Ti ions did not stimulate the activation of inflammatory factors in primary cell cultures. However, higher concentrations of Ti were able to induce a massive release of IL-1β from macrophages, according to the evaluation of human tissues around implants or in physiological solutions [78]. Nonetheless, the expression of inflammatory genes, namely NLRP3, CASP1 and ASC, was not enhanced after exposure to cobalt, chromium, titanium or molybdenum, as evaluated in human primary monocytes [80]. In opposition, Ti particles, alone or under inflammatory conditions, when in contact with fibroblastic and mesenchymal stem cells, evoked a time-dependent increase of ROS production, allied to a decrease of osteogenic differentiation. Furthermore, an *in vivo* study demonstrated evidence of inflammatory effects for metal nanoparticles, with neutrophil and macrophage influx, and upregulation of metalloproteinases in peri-implantitis tissues [14].

Michalkova et al. [89] demonstrated a rapid macrophage polarization by using the RAW 264.7 cell line, after 6 h of exposure to different bundles of anodic TiO_2 nanotubes, besides a dose-dependent increase of hemolysis of human red blood cells. Interestingly, Ti derivatives showed selective cytotoxicity for malignant cells (MDA-MD-231, breast cancer). An innovative system for biomedical implant surfaces based on strontium-loaded sodium titanate nanorods was effective to induce early angiogenesis, *via* induction of M2 macrophage differentiation [90]. Considering the pro-resolution effects of this macrophage phenotype, this nanorod system showed clear advantages relating to the vascularization rates, bone formation and osseointegration. Conversely, it has been recently suggested that macrophages might potentiate the corrosion of a CoCrMo alloy, as proposed as a limiting factor for total hip replacement [91]. In this case, the associated macrophages display a pro-inflammatory phenotype, what likely accounts for the accelerated corrosion rates. It is tempting to extrapolate that alloys present in dental implants can elicit inflammation, that in turn contributes for metal corrosion in a positive feedback basis. Thus, inflammation might present a central role on dental implant failure.

Table 3 – Detailed review on the clinical application of each base-material and the possibilities for surface modifications.

Clinical application	Base-materials	Applied surface modifications	Clinical tissue interaction
Dental implants (intra-osseous)	CpTi (grade I, II,III,IV)	Machined	Oral bone tissue
	Ti-6Al-4V Ti-6Al-4V (ELI) Ti-6Al-7Nb Roxolid (83%–87%Ti-13%–17%Zr)	Polished Anodized Plasma-treated Sand-blasted (particles of Al, Si, Ti, among others)	Gingival tissue
	Zirconia (ZrO_2) Zirconia-toughened alumina	Acid-etched Double acid-etched Laser-treated Functionalized (wettability modification) Coated Biofunctionalized	
Biomedical implants[a]	CpTi (grade I,II,III,IV)	Machined	General bone tissue (all human body regions)
	Ti-6Al-4V Ti-6Al-4V (ELI) Ti-6Al-7Nb Ti-5Al-2.5Fe	Polished Anodized Plasma-treated Sand-blasted (particles of Al, Si, Ti, among others)	General epithelial tissue Gingival tissue
	Ti-29Nb-13Ta-4.6Zr Roxolid (83%–87%Ti-13%–17%Zr) SS, 316 LSS (Stainless Stell) Tantalum Alumina Zirconia (ZrO_2) Zirconia-toughened alumina	Acid-etched Double acid-etched Laser-treated Coated Biofunctionalized	
Trans-gingival components (abutment, healing components, cover)	CpTi (grade I, II, III, IV) Ti-6Al-4V Ti-6Al-4V (ELI) Ti-6Al-7Nb SS, 316 LSS (Stainless Stell) Vitallium, Co–Cr–Mo Au Alloys Tantalum Alumina Zirconia (ZrO_2) Zirconia-toughened alumina PMMA (polymethylmethacrylate) PTFE (polytetrafluoroethylene) PEEK (polyether ether ketone)	Machined Polished Anodized Plasma-treated	Oral bone tissue Gingival tissue Oral epithelial tissue
Prosthetic components (supra-structures)	Ti-6Al-4V	Machined	Gingival tissue
	Ti-6Al-4V (ELI) Ti-6Al-7Nb Ti-5Al-2.5Fe Ti-29Nb-13Ta-4.6Zr Roxolid (83%–87%Ti-13%–17%Zr) SS, 316 LSS (stainless stell) Vitallium, Co–Cr–Mo, Ni–Cr Au alloys Tantalum Alumina Zirconia (ZrO_2) Zirconia-toughened alumina PMMA (polymethylmethacrylate) PTFE (polytetrafluoroethylene) PEEK (polyether ether ketone)	Turned Polished	Oral epithelial tissue

[a] Were considered biomedical implants all metallic-body implants such as maxillofacial implants, plates, mini-implants, orthopedic implants, spinal implants, among others.

Most studies investigating the degradation or release of ions or metal particles analyzed their performance in a short period of time, showing that these chemical elements did not induce toxicity at the initial osseointegration/healing periods [78]. Meanwhile, the current concern is with the transport of chemical elements throughout the body and the possible bioaccumulation, generating unexpected long-term side effects [79]. It is well known that gradual and minimal release by corrosion can induce inflammatory processes such as peri-implantitis, resulting in a gradual loss of osseointegration [14,15,68]. Nonetheless, the systemic impacts of these local reactions cannot be overlooked. Table 3 summarizes all the metallic and ceramic materials applied for implantable components along with your clinical application.

7.1. *Nanotechnology for future and innovative dental implant surfaces*

With the expanded use of nanotechnology and the development of bioactive surfaces, many researchers are focusing its application to minimize the immediate implant infection at the surgical site [92]. For this, the use of materials with inherent antibacterial properties has been explored (*e.g.*, gold, silver, copper, among others) [93]. The presence of these metals was associated with a reduction of biofilm formation, since in many cases the controlled release of metals is extended for more than a week [94]. Most of these studies evaluated the responses of prokaryotic cells to metals at the molecular and morphological levels. However, in most cases, the toxicity of surface-containing metals on eukaryotic cells has been investigated mostly *via* rapid screening protocols, such as the MTT viability test [66]. Thus, in-depth analysis of possible interactions between these micro/nano metallic particles within resident and migrating cells are left without critical and long-term investigation (Fig. 4).

Zhao et al. showed a promising surface functionalized with silver nanoparticles (Ag nanoparticles), demonstrating results of particle release for up to 30 days, with expressive antibacterial properties [95]. Furthermore, high amounts of Ag nanoparticles were associated with significant cytotoxicity after 4 days of culture with rat osteoblastic cells, according to assessment by lactate dehydrogenase (LDH) activity.

These systems depend on both microbial protection and biocompatibility, without one effect impairing the other. Moreover, the human body must be able to degrade or remove the loose chemical elements, without compromising the cells and organs [93,102]. It is well recognized that accumulation of some chemical elements, such as Al, Ag, Cu, among others, may cause different detrimental effects *in vivo* [102,103]. Table 4 recapitulates the "state of the art" in relation to delivery systems incorporated with metallic elements focusing on their possible cytotoxicity or negative inflammatory reactions. Considering the pieces of evidence discussed herein, it is tempting to propose that a broad panel of toxicological assays is required before new biomedical implant devices can enter the market, with a focus on long-term functional studies.

Numerous possibilities of surface functionalization for dental implants have been reported, aiming to improve antibacterial properties, osteogenic responses, antiviral features, immunological responses, among others [93,104–107]. Drug loading on nanotubular surfaces and molecules incorporated in biodegradable materials have been the most explored methodologies in the last 5 years, with the objective of accelerating bone healing or preventing infections [41,105]. Furthermore, modern studies demonstrate the possibility of surfaces modified with antiviral therapies from nanotechnology and drug delivery systems [106], as well as epigenetic functionalization, which could mitigate inflammatory diseases [108]. However, these innovative surfaces are at the preliminary research stage and they still need major developments to reach a clinical market.

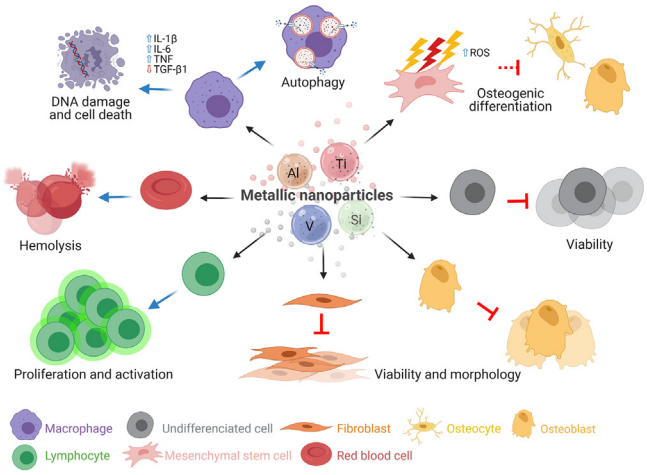

Fig. 4 – The interaction between micro- or nano-particulate metals and biological processes. Metal nanoparticles or impurities might alter physiological cellular pathways, leading to cell damage or death. These materials can also induce inflammatory responses with either local or systemic consequences. These pathways need to be investigated by a complete platform as early as possible during the development on new metal-based nanomaterials.

8. Outlooks and concluding remarks

In the last decades, there were marked advances in the area of dental implants. The current improvements from the pioneer materials are based on the use of different surface treatments and alternative metal alloys. The recent development of innovative metal-based biomaterials was mainly focused on a reduction of total time for functionality recovery, besides a decrease of postoperative infection rates. However, compelling evidence indicates that metals *per se*, their byproducts, or even surface impurities, might act as relevant factors for peri-implant tissue damage. This likely rely on a disruption of tissue homeostasis, *via* induction of local inflammation, as part of the bio-tribocorrosion occurrence. Other concerns are related to the systemic effects and the long-term toxicity of new biomaterials (Fig. 5).

An analysis of literature data makes known that novel studies are required to determine whether metal alloys and different surface treatments can display either local or distant

Table 4 – Studies applying metallic particles with release systems and the response concerning cytotoxicity.

Study	Type of study	Loaded elements	Methodology	Results regarding biocompatibility
Zhao et al. [95]	*In vitro*	Ag nanoparticles	Activity of lactate dehydrogenase (LDH)	A significant cytotoxicity was demonstrated after 4 days in culture with osteoblastic cells in groups with more loaded nanoparticles.
Wang et al. [96]	*In vitro*	Au	No tests	–
Roguska et al. [97]	*In vitro*	Ag and Zn nanoparticles	No tests	–
Yao et al. [94]	*In vitro*	Zn ions	Morphology and proliferation of macrophage-like RAW 264.7 using CCK8 and cell counting	Revealed immunosuppressive effects in 2, 4 and 6 days.
Wang et al. [98]	*In vitro*	Au nanoparticles	Adhesion, proliferation, and ROS detection in MC3T3-E1 osteoblasts	Good cytocompatibility with cells, without generation of intracellular ROS.
Cheng et al. [99]	*In vitro and in vivo*	Strontium (St) and silver (Ag)	Matrix mineralization and gene expression in MC3T3-E1 cells. Also, osseointegration model in rats.	No evident cytotoxic effects were found. The novel surface accelerated the osseointegration process and improved trabecular bone.
Mei et al. [100]	*In vitro* and *in vivo*	Silver (Ag)	Viability, morphology and gene expression of epithelia and fibroblast cell line.	Reported reduction of gene expression and inflammatory response depends of the loaded Ag degree *in vivo*. No rejection or toxic effects were observed.
Yang et al. [101]	*In vitro*	Au nanoparticles	Adhesion, viability, and ALP activity in rat bone mesenchymal stem cells.	Improvement of adhesion, spreading and proliferation of the tested cells.

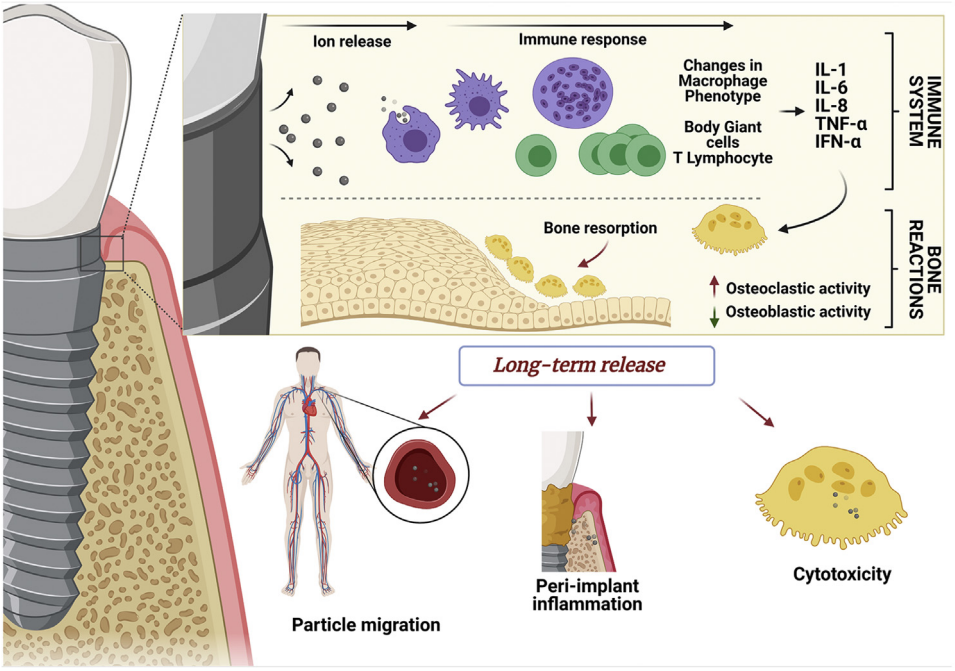

Fig. 5 – Peri-implant tissue responses subsequent to ion release at the biointerface region. Initially, the immune activation might determine an early bone resorption (inbox). Latter, the long-term interactions with ion release might display systemic effects and increased peri-implant disease rates, due to toxicity and/or inflammation.

adverse effects after insertion *in vivo* or in a clinical environment, and to what extent these alterations can compromise the rehabilitation procedure itself, in addition to cause more serious complications in a long-term basis.

The clinical applications of implants highly complicate the possibility of *in situ* analysis in relation to the release of metal ions in adjacent tissues. Toxicity evaluation requires digestion, isolation, characterization and chemical analysis of these

micro-/nanoparticles, what is precluded in a patient with an osseointegrated implant or undergoing clinical treatment. Because of these limitations, some reports have proposed methodologies for simulating the implant insertion processes and *in situ* conditions of an implant in clinical function [109–111], as well as collections of peri-implant fluids for atomic analysis of the region [16]. From these methodologies, it was demonstrated that after installation of implants with modified surfaces, the release of particles and the superficial deformation happened steadily, revealing the impregnation of metallic particles in the model mimicking the bone tissue [110]. In addition, the particles found in these simulation models showed physical–chemical characteristics like those already found in patients' peri-implant tissues [111]. These pieces of evidence substantiate the real significance of the subject proposed in this review.

From a clinical point of view, there is no evidence that the release of ions or the bio-tribocorrosion process are the main factors responsible for the late failure of an osseointegrated dental implant. Usually, the process of losing an osseointegrated implant can be considered multifactorial. However, the presence of metal ions is likely responsible for complex changes in the implant biointerface and adjacent tissues. Alterations in local microbiota and stability of the microenvironment, recurrent inflammation, bone resorption and the presence of peri-implantitis disease have been detailed in the literature [15,68,103,109]. These reports point out the important association of this subject regarding the increased rates of late implant loss and implant contamination under diseases such as peri-implantitis. Based on data discussed in this critical review, it is possible to conclude that rapid advances in implant technologies need to be accompanied by efforts to prove the safety of newly developed biomaterials/surfaces, mainly at pre-clinical levels of investigation and clinical long-term *in situ* follow-ups.

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