UNIVERSIDADE FERNANDO PESSOA

THE ASSOCIATION BETWEEN INFLAMMATION IN PERI-IMPLANTITIS AND BIO-TRIBOCORROSION: SYSTEMATIC REVIEW

[A associação entre a inflamação na peri-implantite e a biotribocorrosão: revisão sistemática]

Dissertação de Mestrado

[Mestrado Integrado em Medicina Dentária]

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Orientador(es):

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ABSTRACT

A systematic review was conducted to evaluate the relationship between titanium metal particles and the development of periimplantitis, specifically: characterization of the inflammatory response regarding cytokine profile in the peri-implant sites, immune cells infiltration and transcription factors up-regulated. This work followed the PRISMA statement, and the literature search was performed, from January 2004 to January 2025, in 3 databases: PubMed, Web of Science and Wiley Library. The inclusion criteria involved articles published in english language, reporting in vivo human studies or in vitro with focus on bio-tribocorrosion of titanium particles around peri-implant soft tissues and their immunological and cellular implications. Quality assessment was based in the Joanna Briggs Institute Critical Appraisal Tools and CONSORT checklist. Of 127 potentially eligible studies, 27 were included: 20 in vitro and 7 in vivo. The median quality assessment score for in vitro studies was 8/14; for in vivo studies, five had no risk of bias and 2 had a moderate risk. There was an increase in the expression levels of IL1-β, TNF-α and IL-6 produced by macrophages, epithelial cells and fibroblasts. In osteoblasts, TNF-α stimulated bone resorption by modulating the RANK-L/OPG balance. There was a significant increase in IL-33 in peri-implant areas associated with higher concentrations of titanium particles and IL-8 production by gingival stromal cells and fibroblasts, regardless of LPS. VEGF was significantly detected in biopsies from patients with peri-implantitis and in peri-implant crevicular fluid, and CCR7 expression decreased in dendritic cells and macrophages. NLRP3, an inflammasome, was up-regulated in response to titanium particles by macrophages, epithelial and mesenchymal stromal cells, and increased levels of DNA methylation were observed in peri-implant tissue. Histological analyses detected mostly neutrophils, macrophages and plasma cells in the peri-implant tissue, and titanium particles were not associated with the presence of multinucleated giant cells. Bio-tribocorrosion of titanium particles stimulates a chronic inflammatory response, activation of the NRLP3 inflammasome and secretion of IL-1β, IL-6 and TNFα in the peri-implant tissue, leading to RANKL/OPG bone resorption. The cytokine IL-33, an alarmin, increased this cellular immune reaction, being constantly produced in response to implant-pillar micromovements. In addition, the production of IL-8 induced by titanium particles attracts neutrophils and monocytes to the site and also acts directly on osteoclasts, causing bone loss. The negative regulation of CCR7 may explain the altered leukocyte migration and the mixture of M1/M2 macrophage populations observed in the granular tissue, ultimately affecting the balance between a successful or failed implant. As far as epigenetic changes are concerned, titanium particles appear to induce DNA methylation, but more studies are needed. Titanium particles are detected in peri-implant tissue in the absence of multinucleated giant cells, suggesting that titanium-induced foreign body reaction is not one of the pathways for osteolysis in peri-implantitis. In the future, immune cell composition and cytokine secretion may help to develop strategies for therapeutic approaches to peri-implantitis, modulating the immune response and helping to prevent implant failure, specifically with the use of biomarkers.

Keywords: dental implants; titanium particles; titanium ions; corrosion; peri-implantitis.

RESUMO

Foi realizada uma revisão sistemática para avaliar a relação entre partículas metálicas de titânio e o desenvolvimento de peri-implantite, mais especificamente: a caracterização da resposta inflamatória traduzida no perfil de citoquinas produzidas nos tecidos peri-implantares, infiltração de células imunitárias e fatores de transcrição regulados positivamente. Este trabalho seguiu a declaração PRISMA, e a pesquisa bibliográfica foi realizada de Janeiro de 2004 a Janeiro de 2025 em três bases de dados*: PubMed*, *Web of Science e Wiley Library*. Os critérios de inclusão envolveram artigos publicados em língua inglesa, relatando estudos in vivo, em humanos, e in vitro com foco na biotribocorrosão de partículas de titânio nos tecidos moles peri-implantares e suas implicações imunológicas e celulares. A avaliação de qualidade foi baseada nas Ferramentas de Avaliação Crítica do *Joanna Briggs Institute* e na lista de verificação CONSORT. Dos 127 estudos potencialmente elegíveis, 27 foram incluídos: 20 in vitro e 7 in vivo. A pontuação média da avaliação de qualidade para os estudos in vitro foi de 8/14; para os estudos in vivo, cinco não apresentaram risco de viés e 2 apresentaram risco moderado. Houve um aumento nos níveis de expressão de IL1-β, TNF-α e IL-6 produzidos por macrófagos, células epiteliais e fibroblastos. Nos osteoblastos, o TNF-α estimulou a reabsorção óssea, modulando o equilíbrio RANK-L/OPG. Houve um aumento significativo de IL-33 em áreas peri-implantares associado a concentrações mais elevadas de partículas de titânio e produção de IL-8 por células estromais gengivais e fibroblastos, independentemente do LPS. O VEGF foi detetado significativamente em biópsias de pacientes com peri-implantite e no fluido crevicular peri-implantar e a expressão de CCR7 diminuiu em células dendríticas e macrófagos. O NLRP3, um inflamassoma, foi regulado positivamente, em resposta às partículas de titânio por macrófagos, células estromais epiteliais e mesenquimais, e foram observados níveis aumentados de metilação do ADN no tecido peri-implantar. Análises histológicas detetaram, maioritariamente, neutrófilos, macrófagos e células plasmáticas no tecido peri-implantar, e as partículas de titânio não foram associadas à presença de células gigantes multinucleadas. A biotribocorrosão das partículas de titânio estimula uma resposta inflamatória crónica, a ativação do inflamassoma NRLP3 e a secreção de IL-1β, IL-6 e TNF-α no tecido peri-implantar, levando à reabsorção óssea RANKL/OPG. A citoquina IL-33, uma alarmina, aumentou esta reação imune celular, sendo constantemente produzida em resposta aos micromovimentos do implante-pilar. Além disso, a produção de IL-8 induzida por partículas de titânio atrai ao local neutrófilos e monócitos e também atua diretamente nos osteoclastos, causando perda óssea. A regulação negativa do CCR7 pode explicar a migração leucocitária alterada e a mistura de populações de macrófagos M1/M2 observada no tecido granular acabando por afetar o equilíbrio entre um implante bem-sucedido ou falhado. No que diz respeito às alterações epigenéticas, as partículas de titânio parecem induzir a metilação do ADN, sendo necessário mais estudos. As partículas de titânio são detetadas no tecido peri-implantar na ausência de células gigantes multinucleadas, sugerindo que a reação a corpos estranhos induzida pelo titânio não é uma das vias para a osteólise na peri-implantite. No futuro, a composição celular imunitária e a secreção de citoquinas podem ajudar a desenvolver estratégias para abordagens terapêuticas à peri-implantite, modulando a resposta imune e ajudando a prevenir a falha do implante, especificamente com o uso de biomarcadores.

Palavras Chave: implantes dentários; partículas de titânio; iões de titânio; corrosão; peri-implantite.

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LIST OF ABBREVIATIONS, ACRONYMS, SYMBOLS, OR ACRONYMS

AIM2	Interferon-inducible protein – absent in melanoma 2
CASP-1	Caspase 1
CASP-3	Caspase 3
CASP-9	Caspase 9
CCL3	C-C motif chemokine ligand 3
CCR7	C-C chemokine receptor 7
CD86	Cluster of differentiation 86
COL-1	Type I collagen
COX-2	Cyclooxygenase-2
CpTi	Commercially pure titanium
DC	Dendritic cell
ELISA	Enzyme-linked immunosorbent assay
FBR	Foreign body reaction
IHC	Immunohistochemistry
IL-10	Interleukin 10
IL-18	Interleukin 18
IL-1β	Interleukin 1-beta
IL-33	Interleukin 33
IL-6	Interleukin 6
IL-8	Interleukin 8
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MNGC	Multinucleated giant cell

MSCs	Mesenchymal stromal cells
NF-kB	Nuclear Factor Kappa-B
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
OPG	Osteoprotegerin
PD	Periodontitis
PI	Peri-implantitis
PICF	Peri-implant crevicular fluid
PIM	Peri-implant mucositis
PIT	Peri-implant tissue
RANKL	Receptor activator of Nuclear Factor kappa Beta
ROS	Reactive Oxygen Species
TGF-β1	Transforming growth factor beta 1
THP-1	Human leukaemia monocytic cell line
Ti6Al4V	Alpha-beta titanium alloy
TiO2	Titanium dioxide
TLR-4	Toll-like receptor 4
TNF-α	Tumour Necrosis Factor Alpha
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

1.1. Dental Implants

Modern implantology originated thanks to the studies of Branemårk during the 1960s, when the orthopaedic surgeon discovered during an experiment how hard bone tissue grew and adhered in the vicinity and contact with titanium. From here, the term 'osseointegration' was coined shortly after.

Interest was focused on the use of orthopaedic prostheses and especially in the sector of oral care where research had advanced in previous decades, providing further improvements in the materials and techniques used in dental implantology (Gaviria et al., 2014).

An important emphasis of this research is the material and shape of dental implants, with most implants currently having a conical or cylindrical root form. This highlights how the design selected for the manufacture has a crucial impact on the outcome of the rehabilitation not only to favour the osteointegration process but also to guarantee a good level of primary stability (Javed et al., 2013; Steigenga et al., 2003). Furthermore, research on the modification of the implant surface, is considered also a fundamental aspect for obtaining and maintaining correct osteointegration, accelerating the bone healing process and promoting a more intimate adhesion (Kieswetter et al., 1996).

Nowadays, the use of dental implants has increased with tooth loss and edentulism, and as an alternative to mobile prosthetic rehabilitation becoming a common practice in dental rehabilitation. In an ageing population it proves to be a reliable technic with a predictable outcome (Noronha Oliveira et al., 2018). In fact, it is estimated that the survival rate of rehabilitation by dental implants is around 90-95% in five years (Berglundh et al., 2002).

Nevertheless, it is important to distinguish between what is defined as success and what is attributed as an index of survival. In fact, it can be deduced that we could consider a dental implant with a good survival rate when it is not affected by mobility and is well anchored to the surrounding bone tissue. At the same time, it could be considered a failure if it shows constant symptoms and signs of inflammation or for example if it partially

exposes the coils that compose the endosseous part, in the oral cavity. Indeed, it has been demonstrated that although it is a rather common practice with a good safety profile, it is not free of limitations and complications from both a mechanical and biological perspective (Albrektsson & Donos, 2012; Zitzmann & Berglundh, 2008).

Has been estimated through case studies that approximately 5 to 11% of dental implants fail during the following 10-15 years (Hermann et al., 2001). The causes that negatively favour the longevity of dental implants have been investigated over the years by numerous authors. Regarding biological factors, two pathological entities have been widely described: peri-implant mucositis (PIM) and peri-implantitis (PI), inflammatory disorders associated with microbiological influences (Lang & Berglundh, 2011). Both conditions initially involve the soft tissues around the osseointegrated dental implant and then culminate in a non-reversable process in which the supporting crestal bone is lost through the reabsorption process, (Lindhe & Meyle, 2008).

1.2 Peri-implant inflammatory diseases

PI is described as a multifactorial inflammatory manifestation mediated by the accumulation of biofilm that affects the soft tissue around the dental implant, a phase described as PIM. If untreated, It can expand to bone tissue causing the loss of the dental implant, although the clear distinction between the two inflammatory conditions is not yet entirely clear. (Lafaurie et al., 2017; Zitzmann et al., 2001).

Recently, a systematic review observed that the prevalence of PI is 20% at patient level, while at dental implant level, it has been estimated to be around 11.5%. It is important to consider that the prevalence undergoes considerable variations, and this is mainly due to the diagnostic criteria used and the variety of the disease definition; thus, leading to a challenge in dental care, given the increase access to dental implants rehabilitation (Diaz et al., 2022).

The consensus for the diagnostic criteria currently includes increased probing depth, bleeding suppuration on probing, and supporting bone loss. More recently, has been established that in the absence of bone loss as determined by radiographic imaging, probing depth alone is not a reliable means of diagnosis lacking sensitivity to detect PI early stages (Albrektsson et al., 2016; Lang & Berglundh, 2011). Although it's suggested

that PI generally appears after few years of dental implant function with his prosthetic component there is a need for early diagnostic biomarkers (Renvert et al., 2018).

Additionally, progression of PI appears to be faster than periodontitis (PD), suggesting a clear distinction between the two pathological conditions; moreover, to date there is no 'gold standard' protocol for the treatment of PI, due to inconsistent and unpredictable results of non-surgical treatments, while only marginal gains are obtained through surgical treatment (Faggion et al., 2013; Keeve et al., 2019).

Regarding risk factors, several studies have been performed to assess and clarify the causes and risk of developing PI: previous history of PD, diabetes, smoking, poor oral hygiene and inadequate plaque control (Schwarz et al., 2018). Although, the exacerbated pro-inflammatory microenvironment results directly from bacterial plaque it can be affected by several other factors like titanium bio-tribocorrosion, implant-abutment micromovements, occlusal overload and cement remnants (Anitua et al. 2023). In the last years, the attention has turned on the incidence of metal debris resulting from titanium wear found in peri-implant soft tissues. A study analysing biopsies from 36 PI soft tissues, detected inflammatory infiltrates and the presence of titanium particles, visible through light microscopy or scanning electron microscopy. Furthermore, exfoliative cytology revealed the presence of metallic particles, attributable to titanium, observing a greater concentration of metallic particles in the extracted samples from patients suffering PI compared to subjects with healthy dental implants, both inside and outside epithelial cells and macrophages, suggesting that the deterioration of the titanium dioxide (TiO2) layer would lead to inflammatory reactions in the surrounding biological tissues (Olmedo et al., 2013; Wilson et al., 2015).

1.3 TiO2 and Bio-tribocorrosion

It is critical to ensure the best biocompatibility and a good compromise from a mechanical point of view; titanium has proven to be the metal of choice for the construction of these devices. The reason for its high level of biocompatibility, as titanium is a highly reactive metal, is largely due to the thin layer of TiO2 spontaneously developed around the implant when the device is exposed to air. This layer of TiO2, therefore, plays a fundamental role in the material biocompatibility, as it avoids direct contact between the metal and the surrounding biological environment, thus reducing the reactivity of the material on the

one hand and, on the other, reducing corrosion (Kheder et al., 2023). Furthermore, it has been demonstrated, in vitro, that the TiO2 layer promotes the deposition of hydroxyapatite on the endosseous surface of the dental implant, thus implementing the osseointegration process (Kodama et al., 2009).

Recently, several factors have been hypothesised influencing the deterioration of the TiO2 layer, through tribocorrosion, term derived from 'tribology' science, that investigates the deterioration of materials subjected to environmental interactions like friction, wear and lubrication. These factors include the type of metal used, suggesting that the use of the alloy Ti6Al4V shows a higher susceptibility to the action of tribocorrosion compared to commercially pure titanium (CpTi), as the formation of the oxide layer would occur in smaller quantities (Gaur et al., 2022).

Furthermore, fluctuations in salivary pH, due in part to dietary factors, pharmacological treatments and bacterial metabolism in the buccal cavity, play a major role in the tribocorrosion process of the osseointegrated device. Also, the fluoride ions contained in toothpastes and mouthwashes interacts negatively with the maintenance of the outer layer of TiO2, exerting a corrosive effect on the implant surface (Apaza-Bedoya et al., 2017). Another described mechanism, central for tribocorrosion of the metal dental implant, is defined as fretting corrosion, resulting from the establishment of micro-movements that occur in the implant/abutment connection during masticatory cycles. This promotes the deterioration and wear of materials, resulting in the release of particles in the biological oral milieu (Swaminathan & Gilbert, 2012).

Regarding the oral microbiome, biological compatibility of the dental implant is also determined by the balance of the oral biofilm. An in vitro study has shown how bacterial colonisation on the dental implant surface, negatively influences the properties of titanium and that, in particular, the late colonising species through the production of organic acids, including lipopolysaccharide (LPS), cause the degradation of TiO2, accelerate the corrosion process and the release of ions and metallic particles (Sridhar et al., 2018).

Recently, a relationship has been hypothesised between tribocorrosion, with particular attention to metallic particles, and the pathogenesis and progression of inflammatory disorders involving peri-implant tissues. Of note, the fact that *S. Mutans*, primary

colonisers of dental implant surfaces, are not present in PD, suggesting a specific bacterial habitat in the implant environment. On the other hand, *P. Gingivalis* which is associated with PI development has greater adhesion when the implant surface already presents a high degree of corrosion. The particles detached by the corrosive action act as a secondary stimulus for inflammation and are partially involved in the monocytic migration process and in osteoclastic proliferation and differentiation (Wachi et al., 2015).

Furthermore, an upregulation of pro-inflammatory cytokines is reported, attributable to the debris detached from dental implants, independently of the action of biological components like LPS. Thus, titanium particles by itself can generate an inflammatory reaction (Quabius et al., 2012). Furthermore, it is confirmed the presence of inflammatory infiltrates in both healthy and failed implants, mainly macrophages and lymphocytes in peri-implant soft tissues (Kheder et al., 2023). The products of material degradation, nano and micro particles promote the activation of the immune system by acting as foreign bodies; many inflammatory mediators and cytokines are involved in this process, as evidenced by titanium particles with a size between 0.25 and 7 μm. They induce the expression of IL-6, TNF-α and IL-1β when in direct contact with macrophages, in particular as a consequence of the secretion of IL-1β, resulting in an imbalance in the rate of bone remodelling due to an increase in RANKL expression (Messous et al., 2021).

Also, the titanium particles induce an increase in the synthesis of reactive oxygen species (ROS) in a time-dependent relationship. The particles are internalised through endocytosis activating cells of the surrounding soft tissue like dendritic cells (DC), macrophage, fibroblasts and epithelial cells causing an abnormal migration of polymorphonuclear cells to the site and exacerbating the local inflammatory response (Bressan et al., 2019).

1.4 Objective of Review

The fundamental aim of this project is to study the relationship between titanium metal particles and the development of PI. We defined the main outcomes as the characterization of the inflammatory response regarding cytokine profile in the peri-implant sites, immune cells infiltration and transcription factors up-regulated.

2. METHODOLOGY

2.1. Materials and methods

This review observed the **Preferred Reporting Items for Systematic reviews (PRISMA)** guidelines and was registered in PROSPERO under the number: CRD42024608114. An electronic search was performed regarding current dental literature in the following databases “PubMed”, “Web of Science” and “Wiley Online Library”. The research was performed during the months of October, November and December 2024, and a filter was applied to incorporate research’s released between the years 2004 and 2025; in addition, only English-language results were considered. The studies that were found using the pre-defined search strategy were independently evaluated according to title and abstract to select the studies that fulfilled the inclusion criteria.

2.2. Review Question

The review focused on the search for current evidence regarding the contribution of bio-tribocorrosion to inflammation in dental implants, with greater emphasis on the wear of titanium particles and ions. More specifically, the main objective of this review was to clarify in which manner titanium metal particles can influence the progression and pathogenesis of peri-implant inflammatory disorders; in this regard, the main Outcome concerns the quantification of inflammatory cells present in situ (macrophages, polymorphonuclear cells and lymphocytes); the analysis and characterisation of inflammatory cytokines, in particular IL-1β, IL-33 and IL-6 and the up-regulation of inflammatory transcription factors. An additional outcome was the detection of titanium particles in tissues and/or cells from failed dental implants. For this purpose, the PECO criteria for reporting the results of the systematic review were implemented. (cf. Table 1)

Table 1

PECO strategy for formulating the research question

P(Population)	Human observational studies and experimental assays (in vitro)
E(Exposure)	Effect of titanium particles resulting from bio-tribocorrosion in peri-implantitis inflammatory reaction
C(Comparison)	Titanium dental implants without inflammatory signs;
O(Outcomes)	Inflammatory response and cytokine production; cell activation and viability

2.3. Screening Process

The systematic review search strategy included both MeSH terms- dental implants "[Title/Abstract]", peri-implantitis "[Title/Abstract]", corrosion "[Title/Abstract]", and relevant keywords - titanium particles "[Title/Abstract]", titanium ions "[Title/Abstract]", to ensure comprehensive coverage of the literature. Boolean operators ("AND" and "OR") were used to relate the above terms to each other.

It should be noted that for each database two independent searches were conducted with the listed combinations, this was carried out to maximize retrieval, which were exported to the Zotero platform for reference management. PRISMA (figure 1) guidelines were adopted for the purpose of writing the body of the manuscript, consisting of a flow chart.

2.4. Eligibility Criteria

The articles that have been included concern the effect of bio-tribocorrosion of titanium particles around peri-implant soft tissues and their immunological and cellular implications. Full-text and open access articles were considered for the research, only findings and reports in English were analyzed for the preparation of this document and the criteria used for the screening process are described below: (cf. Table 2)

Table 2

Inclusion and Exclusion Criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
In vivo studies in humans, such as cohort studies, cross-sectional investigations, case-control analyses and randomised trials have been incorporated	In vivo animal studies
In vitro studies	Studies concerning the wear of titanium particles in organs other than the oral cavity
	Studies in which titanium particles resulted from debridement treatments (implantoplasty and implant scaling)
	Studies with focus on evaluating the effects of implant-abutment connection compatibility and cover screw design on peri-implant disease
	Review papers, correspondence, personal viewpoints, book sections, conference proceeding and summaries.

2.5. Data Extraction

Data were extracted independently from the articles by two reviewers (MF and SS) into evidence tables. Inter-reviewer confidentiality was strictly ensured during the preparation of the manuscript, as each reviewer was independently assigned to specific methodological domain to avoid any potential influence or bias. The subsequent data was obtained: study features (first author's name and year); study design; titanium particles used (size /concentration); Cell line/Model; Inflammatory Biomarkers and Assay type. Finally, a summary of the results was done concerning the major findings.

From the three databases, after an exhaustive search, 661 results were found, specifically 271 from PubMed, 241 open access from Web of Science and 144 open access from Wiley Online Library. These results were converted to BibTex format and processed through Zotero, where 95 duplicates were identified and discarded. 566 articles were

analysed, and 436 results were discarded after evaluation of title and abstract; of the lasting 130 articles, 79 were removed after being examined to determine eligibility based on inclusion and exclusion requirements and 3 articles have been removed as not retrievable. Also, 15 studies were excluded relating metal corrosion mechanisms without considering the biological effects, and finally 12 articles were removed because they were not in line with the systematic review objective. In the end, 6 articles were added manually to the present study as they concerned the revision topic, using a previous review article as a reference. A total of 27 articles were selected for integral reading, of which 20 were in vitro studies and 7 in vivo. Information was independently collected from the articles by two evaluators (MF and SS) into evidence tables:

- Author, Year
- Ti Particles /size
- Cell Line /model
- Inflammatory Biomarker
- Assay
- Summary

The article selection process is specified in the PRISMA flow diagram – Figure 1

2.6. Quality Assessment (QA)

The quality assessment (QA) on the included publications was carried out by two independent reviewers (MF and SS) and any disagreement was resolved through discussion with a third reviewer (JM). Each bias risk analysis was conducted blindly among the reviewers, and only at the end of the assessment process was the final decision made unanimously.

In this regard, it was agreed that in vivo studies were subjected to qualitative analysis following the JBI (Joanna Briggs Institute Critical Appraisal Tools) checklists. Reports were thus divided based on typology, and each study was analysed considering the appropriate checklist. Eight questions are included for cross-sectional studies and ten questions for case-control studies; (outcome of the verified reports are shown in Table 3 and 4; checklists referring to the JBI questions are available in the annexes section).

For both checklists, the risk of bias was classified as "<50%=high risk"; "50 up to 69%= moderate risk" and ">70%=low risk" when the study achieved a "yes" percentage score respectively. (The results of the verified studies are shown in Tables 3 and 4).

To evaluate the methodological rigor of the in vitro investigations, it was implemented the Modified CONSORT checklist identified as a useful tool for issues related to dentistry proposed by Faggion 2012: "Guidelines for Reporting Pre-clinical In Vitro Studies on Dental Materials". This checklist contains 14 items enabling the assessment of each study concerning standard criteria: Background, Objectives, Intervention, Outcomes and Sample size. Then concerning randomization: Sequence generation, Allocation concealment mechanism, Implementation, Blinding and Statistical Methods. Finally, Outcomes, Limitations, Funding and Protocol. For each item correctly reported, it was marked YES and if not NO. The YES score of 14 indicates higher quality. (The results of the QA are shown in Table 5 and 6).

Figure 1

Fluoxogram PRISMA (Preferred Reporting Items for Systematic Reviewsand Meta-Analyses).

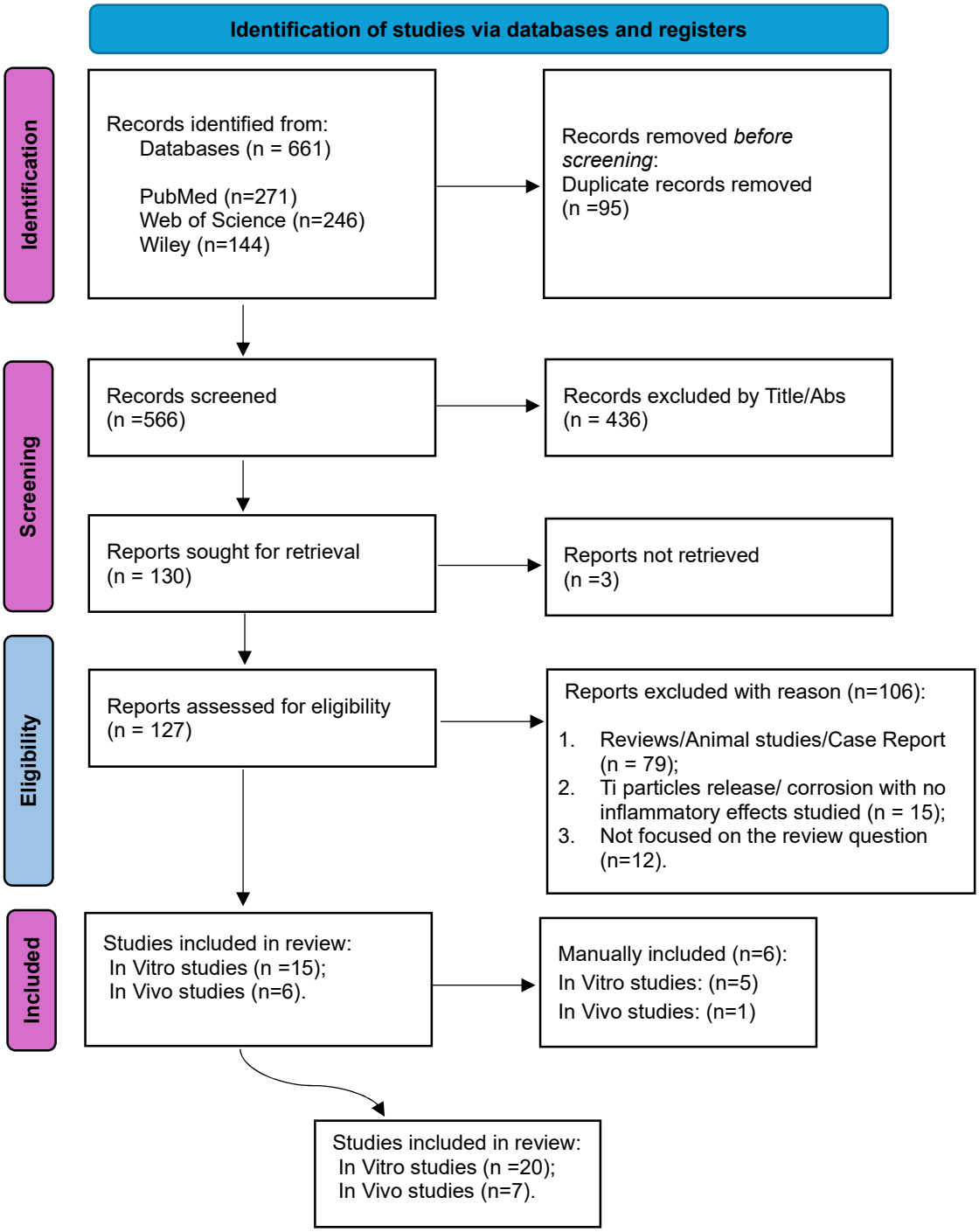


Table 3

Risk of bias - Methodological evaluation of cross-sectional studies - Joanna Briggs Institute Critical Appraisal tools.

Transversal Studies	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	%YES	Risk of Bias
(Rakic et al., 2024)	Y	Y	Y	Y	Y	Y	Y	Y	100	NO RISK
(Stolzer et al., 2022)	Y	Y	Y	Y	Y	Y	Y	Y	100	NO RISK
(Berryman et al., 2020)	Y	N	Y	Y	N	N	Y	Y	62,5	MODERATE
(Wilson et al., 2015)	Y	N	Y	Y	N	N	Y	N	50	MODERATE

Table 4

Risk of bias - Methodological evaluation of case control studies - Joanna Briggs Institute Critical Appraisal tools

Case-control studies	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	%YES	Risk of Bias
(Rakic et al., 2022)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	NO RISK
(Rasul et al., 2021)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	NO RISK
(Daubert et al., 2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	NO RISK

Legend: (Y) Yes, (N) No, (N/A) Non applicable, (N/C) Not clear, (%YES) affirmative answers percentage.

Table 5

Methodological evaluation for In vitro studies CONSORT modified.

ITEMS	Taira et al., 2006	Mine et al., 2009	Chan et al., 2009	Meng et al., 2010	Makihira et al., 2010	Irshad et al., 2013	Mano et al., 2013	Pan et al., 2017	Dodo et al., 2017	Pettersson et al., 2017
1. Abstract	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
2a. Introduction (Background)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
2b. Introduction (Objectives)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
3. Methods (Intervention)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
4. Methods (Outcomes)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
5. Methods (Sample size)	no	no	no	no	no	no	no	no	no	no
6.Methods (Sequence generation)	no	no	no	no	no	no	no	no	no	no
7. Methods (Allocation concealment Mechanism)	no	no	no	no	no	no	no	no	no	no
8. Methods (Implementation)	no	no	no	no	no	no	no	no	no	no

ITEMS	Taira et al., 2006	Mine et al., 2009	Chan et al., 2009	Meng et al., 2010	Makihira et al., 2010	Irshad et al., 2013	Mano et al., 2013	Pan et al., 2017	Dodo et al., 2017	Pettersson et al., 2017
9. Methods (Blinding)	no	no	no	no	no	no	no	no	no	no
10. Methods (Statistical methods)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
11. Results (Outcomes)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
12. Discussion (Limitations)	yes	yes	yes	yes	yes	no	no	no	no	yes
13. (Funding)	yes	no	no	no	no	no	no	yes	yes	yes
14. (Protocol)	no	no	no	no	no	no	no	no	no	no
TOTAL YES	**9**	**8**	**8**	**8**	**8**	**7**	**7**	**8**	**8**	**9**

Table 6

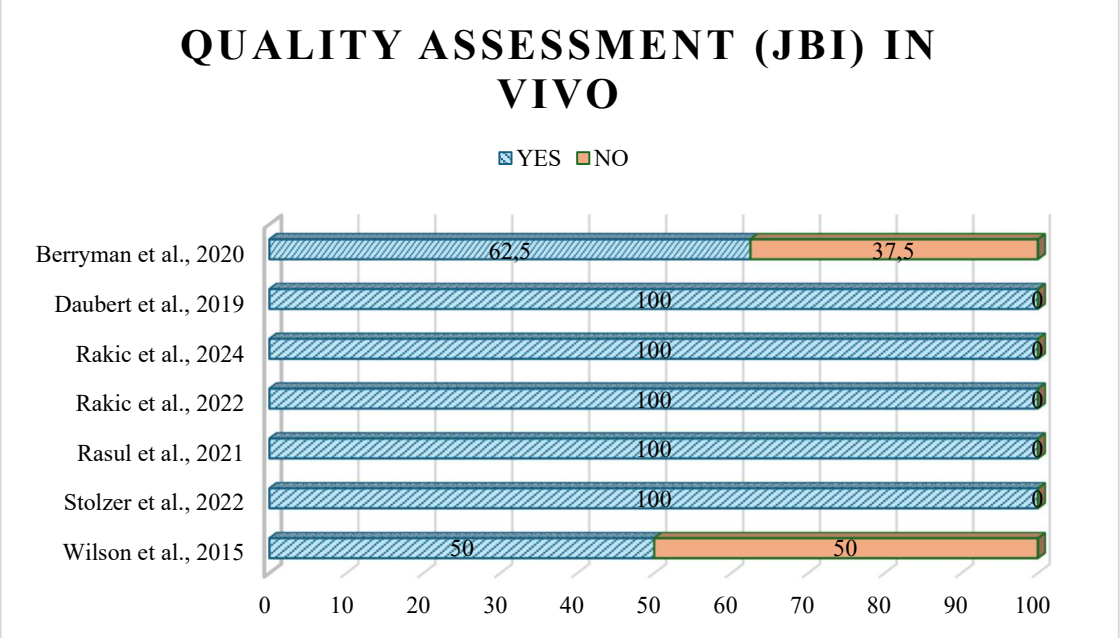
Methodological evaluation In vitro studies CONSORT modified.

ITEMS	Happe et al., 2019	Schwarz et al., 2019	Wang et al., 2020	Li et al., 2021	Callejas et al., 2022	Toledano-Serrabona et al., 2022	Nemec et al., 2022	Papamanoli et al., 2023	Wakuda et al., 2024	Carrillo-Gàlvez et al., 2024
1. Abstract	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
2a. Introduction (Background)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
2b. Introduction (Objectives)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
3. Methods (Intervention)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
4. Methods (Outcomes)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
5. Methods (Sample size)	no	no	no	no	no	no	no	no	no	no
6. Methods (Sequence generation)	no	no	no	no	no	no	no	no	no	no
7. Methods (Allocation concealment Mechanism)	no	no	no	no	no	no	no	no	no	no
8. Methods (Implementation)	no	no	no	no	no	no	no	no	no	no
9. Methods (Blinding)	no	no	no	no	no	no	no	no	no	no

ITEMS	Happe et al., 2019	Schwarz et al., 2019	Wang et al., 2020	Li et al., 2021	Callejas et al., 2022	Toledano-Serrabona et al., 2022	Nemec et al., 2022	Papamanoli et al., 2023	Wakuda et al., 2024	Carrillo-Gàlvez et al., 2024
10. Methods (Statistical methods)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
11. Results (Outcomes)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
12. Discussion (Limitations)	yes	yes	no	yes	yes	yes	yes	yes	yes	yes
13. (Funding)	yes	no	no	yes	yes	yes	no	no	yes	yes
14. (Protocol)	no	no	no	no	no	no	no	no	no	no
TOTAL YES	**9**	**8**	**7**	**9**	**9**	**9**	**8**	**8**	**9**	**9**

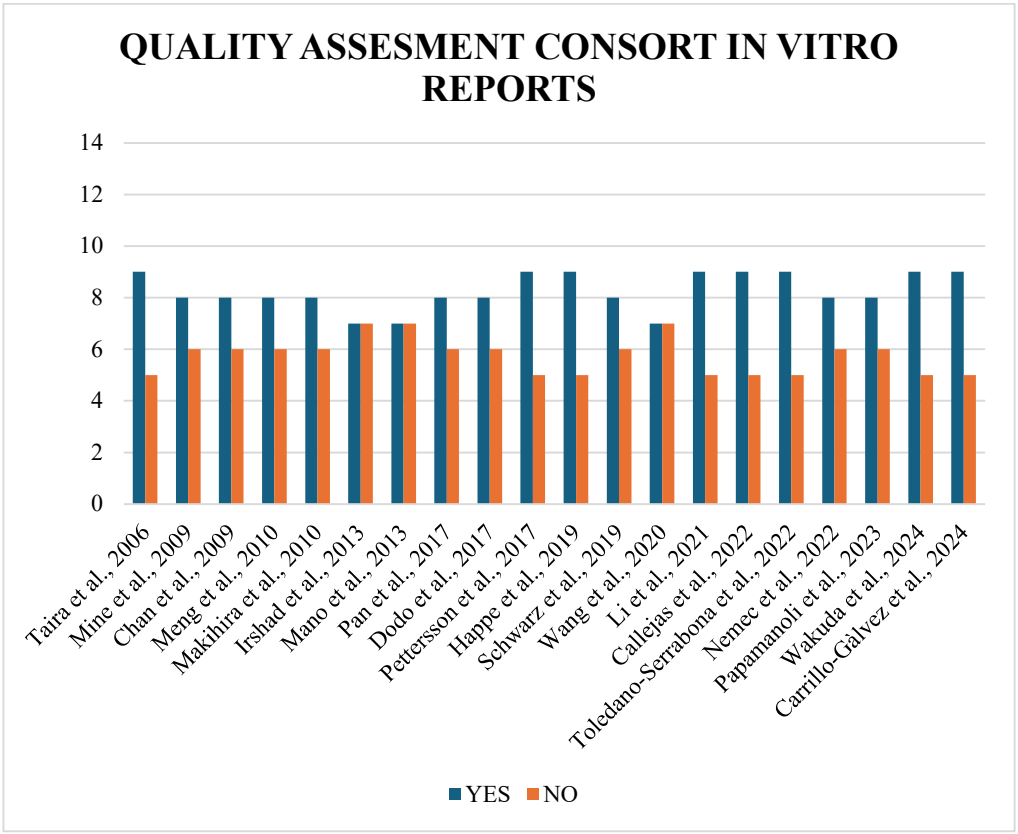
Graphic 1

Summary chart of qualitative analysis results JBI, in vivo studies.



Graphic 2

Summary chart of modified CONSORT qualitative analysis results, in vitro experimental studies.



3. RESULTS

Considering in vivo studies and the JBI quality criteria, five studies had no risk of bias (3 Cross-sectional and 2 Case control), and two papers had a moderate risk of bias (Cross-sectional). Thus, high score achieved denotes that all the studies resulted from strict and structure guidelines and were used to support this review.

Whereas, in relation to the in vitro studies included in the research, the modified CONSORT checklist was used, which has been identified as a useful tool for issues related to dentistry. For the qualitative analysis using CONSORT, have been implemented the guidelines through the paper "Guidelines for Reporting Pre-clinical In Vitro Studies on Dental Materials" proposed by Faggion (Faggion, 2012). (The results of the qualitative analysis are shown in table 5 and 6 and graphic 2).

Considering the key methodology reported to the twenty in vitro studies included, for dental materials used in practice, the median quality assessment score was 8 out of 14. The lowest score was 7 (Irshad et al., 2013; Mano et al., 2013; X. Wang et al., 2016) and the highest score was 9 (Callejas et al., 2022; Carrillo-Gálvez et al., 2024; Happe et al., 2019; Li et al., 2022; Pettersson et al., 2017; Taira et al., 2006; Toledano-Serrabona et al., 2022; Wakuda et al., 2025). It should also be emphasised that only half of the articles provide information regarding external funding. As stated by previous authors (Holliday et al., 2019) all experimental investigations had similar failing domains: "sample size determination, random sequence generation, allocation concealment, implementation details, blinding and publication of the full study protocol." Nevertheless, they were all included in the final discussion

To obtain an overall view of the results, we decided to transfer the information from the reports into a table (cf. Table 7), considering parameters previously defined as important for the proposed review. considering the size of the particles encountered, the cell lines used, and the type of tissue sampled, the inflammatory target of the study, the type of assay performed on the various samples, and finally including a summary of the main results.

Table 7

Characteristics of included studies

IN VITRO STUDIES

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Taira et al. 2006	Ti ions 1 ppm	Macroph.RAW264 (mice)	TNF-α	ELISA	Cultures with Ti show Increased SOD and TNF-α secretion: uptake of Ti-containing complex by macrophages induced oxidative stress and triggered inflammatory response.
Chan et al. 2009	Ti ions (0-100μM)	Dendritic cells blood derived(human)	MHCII, CD80, CD86, CD40, CD54, CD25, CCR4, CCR6 and CCR 7, IL-1β, IL-6, IL-12, TNF-α IL-4, IL-10, TGF-β1, TGF-β2, TGF-β3, CCL17, CCL22	Flow-cytometry, ELISA	Uptake of Ti ions by DCs (membranes, cytoplasm and nucleus). DCs decreased expression of MHCII, CD80, CD86, CD40, CD54, CD25, CCR6 and CCR7 show a weaker immunological synapse with T lymphocytes (only CCR4 slightly increased); Th1 cytokine IL-12 improved substantially as increased lymphocyte stimulatory capacity. In conclusion titanium modifies DC functions, leading to increased T lymphocyte activation and shift towards a Th1-type immune response.
Makihira et al. 2010	Ti ions (1-19 ppm)	Gingival Epithelial (mice)	CCL-2, TLR-4, ICAM-1	RT-PCR	Ti ions increased CCL2 regulation in GE cells subjected to LPS originated from *P. Gingivalis* synergistically; also increased TLR-4 and ICAM-1 expression. This suggests that Ti ions partially contribute to monocyte infiltration in the oral cavity by increasing the responsiveness of gingival epithelial cells to microbial stimuli.
Meng et al. 2010	Ti part 1 μm	Osteoclasts (mice)	TRAP, CAT K, CAII	RT-PCR	Ti particles were phagocytosed; Osteoclast activity enhanced (up TRAP and CAT K) at a lower concentration of Ti particles but inhibited at high.

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Mine et al. 2010	Ti ions (1-9 ppm)	Gingival Epithelial cells (GE), Osteoclasts RAW264.7 and Osteoblasts MC3T3-E1 (all mice)	TRAP, CAT K, RANKL, OPG, Runx2, Osterix, Type I Collagen	RT-PCR	Ti ions at 9 ppm suppressed the expression of Runx2, Osterix and COL-1 in Osteoblasts-like cells and increased RANKL and OPG expression. In osteoclasts no effect in TRAP and cathepsin K (reported previously for this cell line development with RANKL); in GE no effect also for RANK-L and OPG. In conclusion Ti ions impair osteoblast differentiation and modify the RANKL/OPG gene expressions ratio, which is associated with osteoclast differentiation.
Irshad et al. 2013	Ti par (<5um)	Fibroblasts (Human) biopsy	TNF-α, IL-6, IL-1β, IL-8, MCP-1	RT-PCR, ELISA	Ti particles and *P. Gingivalis*, independently, are capable of eliciting pro-inflammatory responses in PIGFs: TNF-α, IL-6 and IL-8 (alone) or TNFα and MCP-1 (combined); Il-1 β induced only by *P. Gingivalis.*
Mano, 2013	Ti par (25nm)	Pulmonary epithelial (NCI-H292) Human	LPS binding protein, CD14, TLR-4, IL-6	RT-PCR, Flow-cytometry	TLR 4, but not LBP or CD 14, plays a role in the internalization of Ti particles to cells and in the inflammatory signal transduction mediated by Ti particles. This last association was seen by the increased IL-6 expression.
Dodo et al. 2017	Ti par(<20um and 21nm)	Macro.THP-1(Human)	TNFα, IL-1 β, IL-6	RT-PCR, ELISA	Ti nano or microparticles and LPS *(P. Gingivalis*) and macrophages- impacted viability and inflammation-gene expression and cytokine release was significantly greater for TNF-α and IL1-β after 12 h, and for IL-6 but only 24 h later. There is robust pro-inflammatory response in nanoparticles independently LPS presence.

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Pan et al. 2017	Ti ions	Fibroblasts L929 (mice)	CASP-9, CASP-3	RT-PCR	Ti particles have a cytotoxic effect on fibroblasts (time-dependent): upregulation of CASP-9 and CASP-3 and apoptosis by the intrinsic pathway. Apoptosis might not be a result from the ions released but from titanium particles compromised cell adhesion, which conducted cell apoptosis by inhibiting the focal adhesion association.
Petterson et al. 2017	Ti ions	Macro.THP-1(Human)	NLRP3, ASC, CASP-1, IL-1β, IL-1a, b, IL-2, IL-4, IL6, IL-8, IL10, IL12, IL-17, IFNγ, TNFα, GM-CSF	RT-PCR, ELISA	Ti ions stimulated inflammasome activation: IL-1β release. LPS exposure enhances this effect. Nevertheless, Ti ions alone did not promote transcription of the inflammasome components. In conclusion Ti ions establish particles that represent a secondary stimulus for a proinflammatory response.
Happe et al. 2019	Ti part <20 µm	Gingival fibroblasts and Osteoblasts (Human)	TNF-α, IL-6	ELISA	Ti particles concentration affects cell growth and proliferation and as cytotoxic effects specially in GF; osteoblasts produce IL-6 only 24 hours after contact, but GF produce IL-6 continuously-21 days. Ti particles were identified inside the cells.
Schwarz et al. 2019	Ti part (60-100nm)	Gingival fibroblasts and Osteoblasts (Human), Macrophage THP-1 (Human)	IL-1β, IL-6	ELISA	Increasing Ti particle concentration correlates negatively with cell viability both in osteoblasts and GF. THP-1 didn´t show a substantial improve in IL-1β and IL-6 concentrations. Thus, no pro-inflammatory effects.

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Toledano-Serrabona et al. 2020	Ti part. (30-70nm)	Macrophage THP-1 (human) and bone marrow-derived mesenchymal stem cells- BM-MSCs (Human)	CCR7, TNF-α, IL-1β, CD206, TGF-β, IL-10	RT-PCR, ELISA	Macrophages stimulated by Ti particles developed an amplified proinflammatory expression of TNF-α and a reduced expression of TGF-β and CD206. Regarding cytokine release, there was an increase in IL-1β, whereas IL-10 was reduced. Ti particles concentration negatively correlates with BM-MSCs cells viability, and the cells showed a significant decrease in Runx2 and OC expression- osteogenic response markers. Ti particles elicit a pro-inflammatory response and suppress osteogenic gene expression.
Wang et al. 2020	Ti ions 10-50 μm	Osteoblasts (Human)	mROS, SOD, SIRT	Western Blot	Ti ions reduced osteoblast capability. With improved Ti ion concentration, the expression levels of LC3 gradually increased, P62 reduced, autophagic flow amplified and mROS levels improved. Also, activity of SOD2 / SIRT 3 decreased.
Callejas et al. 2022	Ti part (5-30 μm)	Macrophage THP-1 (human)	TNFα, IL-1β, CCR7, IL-10, TGF-β, CD206, TNFα, IL-1 β, IL-10	RT-PCR, ELISA	Smaller Ti particle sizes show less cytotoxicity. At non cytotoxic concentrations, TNFα and IL-1β expression, inflammatory indicators, were higher related to bigger Ti particles: particles of 15 μm showed a minor proinflammatory and higher anti-inflammatory reaction as categorized by gene expression and cytokine release: biocompatible and present a lower immune response.
Li et al. 2022	Ti part (<5μm and <100nm)	Gingival fibroblasts (Human)	FAK,fibronectin, COL1	RT-PCR, Western Blot	Ti nanoparticles significantly inhibited GF cell viability, proliferation, and migration compared with microparticles. Also, they caused cytoskeleton disruption as measured by protein expression. This effect was enhanced with LPS.

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Nemec, 2022	Ti part (<100 nm) nano	Gingival mesenchymal stromal cells (Human)	IL-6, IL-8, MCP-1	RT-PCR, ELISA	Cell proliferation and viability were inhibited by Ti nanoparticles (<100 nm). They also elicited strong expression of IL-8, and this response was enhanced by LPS.
Papamanoli, 2023	Ti part (10.4 ± 6.4 μm)	Gingival fibroblasts (Human)	IL-6, IL-8, Col-1a	RT-PCR	Association of Ti particles and LPS substantially increased expression of IL-6, IL-8 and Col-1a. It seems that particles may stimulate comparable reactions to the endotoxin, whereas synergistically amplifying it.
Carrillo-Galvèz et al. 2024	Ti ions	Bone derived mesenchymal stromal cells (Human)	NLRP3, AIM2, IL-1β	RT-PCR, ELISA	There is induction of NLRP3 and absence of AIM2 inflammasome pathways facilitated by bacterial factors, with increased release of IL-1β. When bacterial components, in combination with Ti ions, NLRP3 expression is further enhanced while AIM2 expression is reduced. In conclusion, the progression of inflammation in peri-implantitis may be more critical due to the mutual effect of organic and inorganic elements that enhance NLRP3 inflammasome activation.
Wakuda et al. 2025	Ti par 25 um (30-100ug/ml)	Gingival epithelial (Ca9-22) (Human)	COX2, ROS and TGF-β1, NLRP1, NLPR3, CASP1, IL-1β	RT-PCR, ELISA	Cells treated with Ti particles showed an increase of 75% cell capability through all dilutions. Inflammation-related genes (COX2 and TGF-β1) substantially intensified in a dose-dependent way. NLRP3 and CASP1 expression increased, as well as the secretion of IL-1β. Also, there were improved ROS levels after testing with Ti particles.

IN VIVO IN HUMANS STUDIES

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Wilson et al. 2015	Ti particles	36 peri-implantitis(biopsies)	Chronic inflammatory infiltrates-plasma cells; MGNC	Histological analysis	Chronic inflammatory infiltrates-plasma cells; Multinucleated Giant Cells were observed.
Daubert et al. 2019	Ti particles	21 peri-implantitis, 24 healthy implants (PICF and biopsies)	DNA methylation	ELISA	Increased levels of methylated DNA in peri-implantitis; association of Ti levels and global methylation, independent of peri-implantitis suggesting methylation may be affected by Ti dissolution products.
Rasul et al. 2021	Ti particles	30 peri-implantitis, 20 healthy implants (biopsies)	Peri-implantitis parameters (Probing depth, Plaque index, Gingival index)	Mass spectrometry	Notably higher Ti level in submucosal plaque surrounding dental implants with symptoms of peri-implantitis comparing with healthful dental implants.
Berryman et al. 2022	Ti particles	10 peri-implantitis (biopsies)	TGF-β1, RANKL, IL-33 and CD68	IHC, ELISA	Tissue samples showed a mixed chronic inflammatory infiltrate. Substantial upregulation of cytokine RANKL detected, with a tendency toward overexpression of IL-33 and TGF-β1 in areas with Ti.

Author, Year	Ti Particles /size	Cell Line /model	Inflammatory Biomarker	Assay	Summary
Rakic et al. 2022	Ti particles	39 peri-implantitis ,35 periodontitis (biopsies)	CD68, IL-6, NF-kβ and VEGF	IHC and histological analysis	Neutrophil infiltration inside the granulation matrix, absence of Multinucleated Giant Cells or specific inflammatory patterns; severe neovascularization and persistent immune cell infiltrate mainly composed by plasma cells, neutrophils and macrophages- high CD68 and VEGF
Stolzer et al. 2023	Ti particles	20 peri-implantitis, 20 healthy implants, 20 no implants (peripheric blood)	TNFα, IL-1β	ELISA	Significant relationship of positive Ti stimulation and peri-implantitis clinically and radiologically; macrophages in 28,3% of individuals across all groups emitted proinflammatory cytokines beyond normal biological levels.
Rakic et al. 2024	Ti particles	36 peri-implantitis, 36 peri-implant mucositis, 39 healthy implants,37 periodontitis (PICF and biopsies)	Chronic inflammatory infiltrates, MGNC and VEGF	IHC and histological analysis, ELISA	TPs were detected as unbound material enclosed within granulation tissue, but no Multinucleated Giant Cells or impaired phagocytes observed - no evidence of foreign body response or distinct pathological impact caused by TPs in peri-implantitis. VEGF markedly upregulated in peri-implantitis relative to periodontitis and shows a positive association with its soluble levels in PICF.

After analysing the 27 articles, and centring on the objective of the manuscript, it was decided to summarise the results, categorising them according to critical parameters for a better overall understanding.

It was categorised the size of micrometric and sub-micrometric titanium particles and evaluated their effects on different cell types. The results were then grouped into three areas of interest, considering quantification and evaluation in terms of secretion/expression of interleukins and TNF-α, quantification and evaluation of transcriptional factors, inflammasome and other mediators of inflammation, and finally expression of chemotactic and growth factors. (Tables 8, 9 and 10).

The results shown in Table 8 show the characterization of changes in the expression of TNF-α and interleukins 6, 1β, 8, 12, 10 and 33. In accordance with the results of five in vitro trials, an elevation in TNF-α secretion was reported (Taira et al., 2006; Irshad et al., 2013; Dodo et al., 2017; Callejas et al., 2022; Toledano-Serrabona et al., 2022), in response to exposure to titanium particles. They varied in size from 30 nm to 25 μm and the effect was observed in epithelial cells, macrophages and fibroblasts. One in vivo study evaluating TNF-α expression in macrophages derived from peripheral blood had the same result and also showed IL1-β increase (Stolzer et al., 2023). The later, was in line with five other in vitro studies, 4 in macrophages (Dodo et al., 2017; Pettersson et al., 2017; Callejas et al., 2022; Toledano-Serrabona et al., 2022;) and one in epithelial cells (Wakuda et al., 2025). Furthermore, five in vitro studies supported also an increase in IL-6 expression following exposure to titanium particles ranging in size from < 5 to < 25 μm, in macrophages, fibroblasts and epithelial cells (Dodo et al., 2017; Happe et al., 2019; Irshad et al., 2013; Mano et al., 2013; Papamanoli et al., 2023).

Regarding IL-10, this anti-inflammatory cytokine was evaluated by 4 in vitro studies. Both DCs and macrophages showed a decreed IL-10 expression upon exposure of the cells to titanium ions or particles, respectively (Chan et al., 2009; Toledano-Serrabona et al., 2022). In the two other studies, the cytokine increased when macrophages were exposed to titanium highest particle concentration (30 μm) (Callejas et al., 2022; Pettersson et al., 2017).

Three out of four reports show an increase in IL-8 production by gingival stromal cells and fibroblasts (Irshad et al., 2013; Nemec et al., 2022; Papamanoli et al., 2023) and only one detected IL-12 by stimulating DCs (Chan et al., 2009). Also, there was one study on

IL-33, detecting overexpression both in biopsies and peri-implant tissue (PIT) (Berryman et al., 2020).

Table 9 shows the variations encountered in terms of transcriptional factors, inflammasome formation and other inflammatory markers, as reported by the authors of in vitro and in vivo investigations. The only two studies, in vitro, on TLR-4 expression, confirmed its increase after exposure to titanium ions and particles under 25 nm on gingival and pulmonary epithelial cells (Makihira et al., 2010; Mano et al., 2013). In the only study conducted on epithelial, osteoblast and osteoclast-like cells it was detected an increase in RANKL and OPG expression in osteoblasts, through stimulation with 20 ppm titanium ions (Mine et al., 2010); This outcome was confirmed by the one only in vivo study on the same biomarkers when analyzing ten biopsies of peri-implantitis (Berryman et al., 2020). On the other hand, induction of NLRP3 inflammasome expression was detected in the two studies using titanium particles either in epithelial or mesenchymal stromal cells. In epithelial cells this observation was ROS dependent and in the other cell line this effect was synergetic with bacterial components respectively (Carrillo-Gálvez et al., 2024; Wakuda et al., 2025). In the study using titanium ions and macrophages this effect was not observed by the ions alone, only with LPS and it was associated with an IL-1 β increase (Pettersson et al., 2017).

Regarding caspase activation it was observed a time-dependent cytotoxic effect with increased expression of CASP-9, CASP-3 and CASP-1 in fibroblasts, macrophages and gingival epithelial like cells respectively (Pan et al., 2017; Pettersson et al., 2017; Wakuda et al., 2025).

Lastly, Table 10 illustrates the alterations observed in relation to the expression of chemotactic and growth factors. Looking to the four in vitro studies that assessed TGF-β there are opposite results: Chan and Toledano-Serrabona observe a decreased expression when exposing DCs or macrophages to titanium ions; Callejas and Wakuda observed an increase in TGF- β expression when exposing macrophages or epithelial cells to titanium particles. The latter was observed also in vivo either in biopsies or peri-implant crevicular fluid (PICF) (Berryman et al., 2020). For CCR7 the 3 in vitro studies that evaluated its expression showed a homogeneous result. There was a decrease of this chemokine receptor expression either in DCs or macrophages (Chan et al., 2009; Toledano-Serrabona et al., 2022) . In the other study the same trend was observed only in the presence of *P. Gingivalis.*

In vivo studies show a significant increase in VEGF in tissues derived from patients with PI versus patients with PD and they detected titanium particles in all PI biopsy samples (Rakic et al., 2022, 2024).

Table 8

Interleukin and Tumour Necrosis Factor

IN VITRO STUDIES									
Author, Year	**Ti particles/Ions Size**	**Cell Type**	**TNF-α**	**IL-6**	**IL1-β**	**IL-8**	**IL-12**	**IL-10**	**IL-33**
Taira, 2006	Ions	Mac	↑						
Chan, 2009	Ions	DC					↑	↓	
Irshad, 2013	part.: < 5 µm	Fib	↑	↑		↑			
Mano, 2013	part.: < 25 µm	Epi		↑					
Dodo, 2017	part.: < 21 nm	Mac	↑	↑	↑				
Pettersson, 2017	Ions	Mac			↑			↑	
Happe, 2019	part.: < 20 µm	Fib		↑					
Toledano-Serrabona, 2022	part.: (30-70 nm)	Mac	↑		↑			↓	
Callejas, 2022	part.: 5- 30 µm	Mac	↑		↑			↑*	
Nemec, 2022	part.: (100 nm)	GSC				↑			
Papamanoli, 2023	part.: (15 µm)	Fib		↑		↑			
Wakuda, 2025	part (25 µm)	Epit			↑				
TOTAL			**5**	**5**	**5**	**3**	**1**	**4**	**0**

IN VIVO STUDIES									
Author, Year	**Ti particles/Ions Size**	**Cell/Tissues**	**TNF-α**	**IL-6**	**IL1-β**	**IL-8**	**IL-12**	**IL-10**	**IL-33**
Berryman, 2020	-	PIT Biopsy							↑
Stolzer, 2023	-	PB Mon/Mac	↑		↑				
TOTAL			**1**		**2**				**1**

Notes: Mac, Macrophages; DC, Dendritic Cells; Fib, Fibroblasts; Epit, Epithelial Cell line; GSC, Gingival Stromal Cells; PIT, peri-implant tissue; PB, peripheral blood; Mon, Monocytes; Mac, Macrophages; *only with 30 µm Ti particles

Table 9

Transcriptional factors, Inflammasome and other inflammatory-related markers

IN VITRO STUDIES

Author, Year	Ti Particle/ Ions	Cell Type	TLR4	RANKL	OPG	CASP-1,9,3	NLRP3	AIM2	COX-2
Mine, 2009	ions	GinE Ostb Ostc		↑	↑				
Makihira, 2010	ions	GE-1 Epit	↑						
Mano, 2013	par.: < 25 nm	Epit	↑						
Pan, 2017	ions	Fib				↑			
Pettersson, 2017	ions	Mac				↑	↑		
Carrillo-Gàlvez et al., 2024	ions	HMSC					↑		
Wakuda, 2025	par.: (25 µm)	Epit				↑	↑		↑
TOTAL			**2**	**1**	**1**	**3**	**3**		**1**

IN VIVO STUDIES

Author, Year	Ti Particle/ Ions	Tissues	TLR4	RANKL	OPG	CASP-1,9,3	NLRP3	AIM2	COX-2
Berryman, 2020	-	PIT biopsy		↑					
TOTAL				**1**					

Notes: GinE, Gingival Epithelial cells; Ostb., Osteoblasts; Ostc., Osteoclast; Mac, Macrophages; hMSC, Human Mesenchymal Stem Cells; Fib, fibroblast. PIT, peri-implant tissue; GE-1, gingival epithelial-like cells.

Table 10

Chemokines and Growth Factors

IN VITRO STUDIES						
Author, Year	**Ti Particles/Ions Size**	**Cell Type**	**CCR7**	**MCP-1**	**TGF-B**	**VEGF**
Chan, 2009	Ti ions	DC	↓		↓	
Irshad, 2013	par.: 5µm	Fib		↑		
Toledano-Serrabona, 2022	par.: (30-70 nm)	Mac	↓		↓	
Callejas, 2022	Ti part (5-30 µm)	Mac	↓*		↑	
Nemec, 2022	part.: (100 nm)	GSC		↓		
Wakuda, 2025	par.: (25 µm)	Epit			↑	
TOTAL			**3**	**2**	**4**	
IN VIVO STUDIES						
Berryman, 2020	-	PIT Biopsy			↑	
Rakic, 2022	-	PIT Biopsy				↑
Rakic, 2024	-	PIT Biopsy PICF				↑*
TOTAL					**1**	**2**

Notes: DC, Dendritic Cell; PIT, peri-implant tissue; Fibro, Fibroblasts; Mac, Macrophages. * With LPS; PICF, peri-implant crevicular fluid

4. DISCUSSION

The studies included in this review, both in vivo and in vitro, confirm the association between the inflammatory response in peri-implantitis and the dissolution products of titanium dental implants, i.e. bio-tribocorrosion, depending on the type of particle observed, its size and the time during which the particles or ions interact with the surrounding biological environment.

Considering the association between pro-inflammatory interleukins and exposure to different sizes of titanium particles, the included studies point to an increase in the expression levels of IL1-β, TNF-α and IL-6 produced by macrophages, epithelial cells and fibroblasts (Callejas et al., 2022; Dodo et al., 2017; Happe et al., 2019; Irshad et al., 2013; Papamanoli et al., 2023; Stolzer et al., 2023; Taira et al., 2006; Toledano-Serrabona et al., 2022; Wakuda et al., 2025). These findings agree with several studies indicating the same cytokine increase in PIT either by protein expression or immunohistochemical analysis. Ultimately, TNF-α induces RANKL expression, modulating the balance RANKL/OPG and stimulates bone resorption as observed by Mine when coculturing titanium particles with osteoblasts and also by several other authors (Messous et al., 2021; Mine et al., 2010; Noronha Oliveira et al., 2018; Vallés et al., 2006).

IL-1β, is a pro inflammatory cytokine, produced by several cells like macrophages, neutrophils and DCs with a key role in immune regulation, especially in connective tissue and bone: it inhibits the formation of type I fibrillar collagen and induces osteoclast activation. TNF-α, another pro-inflammatory cytokine produced mainly by macrophages, rapidly after infection as numerous effects like prostaglandin synthesis, cell proliferation and differentiation, tumorigenesis, and induction of osteoclast activation, affecting the balance RANKL/OPG. IL-6 is a pleotropic cytokine, produced by immune cells, fibroblasts, endothelial cells and other, associated with inflammation, immune response, and haematopoiesis. It is produced in response to infection and tissue damage such as trauma, with high levels being detected around failed implants. Previous authors with several studies both in vivo and in vitro showed that IL-6, IL-1β, and TNF-α are up-regulated in PIT, stimulating an inflammatory response with soft tissue destruction and bone resorption, induced by titanium particles and clinically translated in PI and implant failure.

Still looking to the interleukins, an in vivo, cross-sectional study involving ten biopsies from patients with PI found significant upregulation of IL-33 which was more pronounced in tissue areas with higher titanium particle concentrations (Berryman et al., 2020). There is only one study, in vitro, that assessed IL-33 levels in PICF and found that it was increased significantly in PI (Severino et al., 2016). A recently discovered cytokine, IL-33, has been found to play a central role in several diseases, such as rheumatoid arthritis, asthma, Sjogren's syndrome and also PD (Alarcón-Sánchez et al., 2024). IL-33 is abundantly produced by endothelial, epithelial and fibroblast stromal cells in human and mouse tissue. Unlike other cytokines it is already present in normal tissue rising very quickly after endothelial cell damage or mechanical injury, which is why it is called an alarmin (Cayrol & Girard, 2022).

From our results it can be suggested that even before the production of IL-1β, IL-6 and TNF- α triggered by titanium particles phagocytosis, IL-33 is released as a result either from the initial drilling procedure at implant placement that causes bone disruption or later as result of biomechanical stress induced by micromovements at implant-abutment interface. These, associated to functional loading during masticatory activity and the presence of micro gaps, together can provoke repetitive mechanical stimulation of PIT.

In this review it was also observed another trend, the production of IL-8 by both gingival stromal cells and fibroblasts, opposite to previous studies, that mark this chemokine as unaltered in PI (Severino et al., 2016). In fact, by testing the effects of exposure to particles derived from the degradation of titanium, in vitro studies showed an increase in IL-8 production, independent of the biological influence of LPS (Irshad et al., 2013; Nemec et al., 2022; Papamanoli et al., 2023).

IL-8 plays an important role in the recruitment of neutrophils during inflammation, angiogenesis, keratinocyte migration and cell proliferation. Although studies on total hip replacement point out that M1 macrophages can induce IL-8 production and affect the inflammatory response in response to titanium wear particles, this is still controverse for PI (Bijukumar et al., 2020).

Our results reinforce a role for IL-8 in PI as in hip implants and very recently new information by transcriptome analysis of peri-implant gingival tissues showed also an upregulation of IL-8 expression (Kheder et al., 2023).

It was also observed by two in vivo studies that VEGF expression was markedly higher in PI than in PD both in biopsies and PICF. Nevertheless, despite titanium particles detection in granulation tissue from the biopsies a direct effect cannot be infer (Rakic et al., 2022, 2024). VEGF an angiogenic and vasculogenic factor, produced by immune cells, is associated with persistent inflammation and progression of gingivitis to PD (Bertoldo et al., 2024). Although its role in PI is not very clear, it can act independently or in combination with TNF-α enhancing osteointegration and wound healing. In titanium particles induced peri-prosthetic osteolysis, VEGF was proposed as a biomarker. In PI, an infection associated inflammation, VEGF presence in samples from PICF of patients with dental implants make it also a predictable biomarker of PI; nevertheless, there is no histopathological pattern associating titanium particles presence and VEGF and further studies are necessary.

Regarding the inflammasome NLRP3, an innate protein receptor, downstream of TLR-4, regulator of CASP-1 and inflammatory response to infectious agents, it was observed an up-regulation, in response to titanium particles by macrophage, epithelial and mesenchymal stromal cells. This cellular response was paralleled by IL-1β production and enhanced by LPS (Carrillo-Gálvez et al., 2024; Pettersson et al., 2017; Wakuda et al., 2025). These are the first in vitro reports of NRLP3 activation by titanium particles, specifically in PI. One work in human PI biopsies observed increased expression of NLRP3 /AIM2 inflammasomes, CASP-1, and IL-1β, and, in mice, NRLP3 inflammasome upregulation induced periapical lesions. Thus, neither accessed the cellular response to the presence/absence of titanium wear debris (Galindo-Moreno et al., 2023; Zhu & Liu, 2022). It is clearer that both bacterial and titanium/metal particles can activate innate immune cells in PI and cause IL-1β dependent- osteolysis. Thus NRLP-3 could be an efficient diagnosis biomarker.

Also looking to chemotactic factors, for the first time, a decrease in CCR7 is emphasised. Firstly, a study conducted on DCs showed that, following exposure to titanium ions, a significant reduction in the expression of CCR7 was observed in addition to various molecules such as CD40, CD54, CD80 and CD86 (Chan et al., 2009). A decrease in CCR7 expression can impair normal DC movement leading to chronical inflammation. Also, in macrophages it was observed a significant decrease in CCR7 expression, tested either alone or with LPS respectively (Callejas et al., 2022; Toledano-Serrabona et al., 2022). In macrophages this result can reflect a reduced migration capacity but also a shift

in macrophage phenotype from M1 to M2. In the first study this is backed up by the common decrease of CCR7 and the anti-inflammatory markers TGF-β and IL-10. In the second study by Callejas and colleagues the decreased was also observed except with the larger titanium particles. Thus, in PI there is a mixed M1 and M2 population that can be helpful when testing PI treatments, directing the immune response towards M2 polarization (Chato-Astrain et al., 2024).

Regarding the cytotoxic effect of titanium debris, in this review, for the first time, a study quantifying DNA methylation both in PICF and biopsies, showed that the group with PI had higher levels of methylation (5mC) compared to the control group. This result was further enhanced in tissues adjacent to titanium particles. Thus, epigenetic fluctuations and global DNA methylation is closely linked to the presence of titanium particles in PI (Daubert et al., 2019). However, recent works on PI "molecular signatures" and epigenome were more cautious associating epigenetic changes in oral cells treated with titanium particles (Freitag et al., 2023). This latest finding leads us to hypothesise that epigenetic variations can be explained non only by the cellular interaction with titanium particles but by a wider interaction with organic compounds in the environmental medium.

Histology of the PI biopsies showed a mix chronic inflammatory infiltrate of neutrophils, macrophages and plasma cells consistent with chronic inflammation. Regarding foreign body reaction (FBR) recent studies did not detect the presence of multinucleated giant cells (MGNC) in biopsies from PI patients (Rakic et al., 2022, 2024). In the other in vivo human study, Wilson and colleagues observed this presence in 34 out of 36 biopsies of PI although only in 7 it was confirmed the presence of titanium particles (Wilson et al, 2017). MGNC result from macrophage fusion and are commonly seen in response to biomaterials. Their presence can indicate titanium particle phagocytosis depending on the size: less than 25 μm. They can be associated with a pro-inflammatory response but also with wound-healing after chronic inflammation. (Ivanovski et al, 2022; Insua et al, 2024).

Altogether, these findings suggest that FBR is not a result of titanium wear and a secondary cause of implant failure. It does not provide sufficient evidence to be considered a risk factor for implant rehabilitation failure.

The studies included in this review confirm previous ones showing that titanium particles can have a direct impact on implant bone resorption, affecting the osteoblast/osteoclast

balance, but also an indirect action, causing an exacerbated pro-inflammatory response which will also lead to osteolysis. Our studies suggest that the later may be reflected in cellular alterations in the peri-implant tissues in the production of cytokines, the activation of 'alarm' proteins and cell migration in response to a 'non-infectious' agent, which in a positive feedback loop, contributes to implant failure.

5. CONCLUSION

The immune system plays a crucial role in maintaining homeostasis particularly during implant insertion. After implantation it enables wound healing, prevents infection and controls the inflammatory process underlying bone formation and resorption. Thus, excessive inflammation of the soft surrounding tissue can be exacerbated by titanium particles dissolution subsequent to bio tribocorrosion and leading to implant failure. It is thought that characterizing the inflammatory response in PI, the cellular composition, cytokine secretion and up-regulation of transcription factors may help in designing strategies for therapeutic approaches or for implant failure prevention profiling risk patients and immune biomarkers of PI.

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ANNEXES

Annex A. JBI checklist

Cross-sectional studies (JBI checklist):	
Q1.	Were the criteria for inclusion in the sample clearly defined?
Q2.	Were the study subjects and the setting described in detail?
Q3.	Was the exposure measured in a valid and reliable way?
Q4.	Were objective, standard criteria used for measurement of the condition?
Q5.	Were confounding factors identified?
Q6.	Were strategies to deal with confounding factors stated?
Q7.	Were the outcomes measured in a valid and reliable way?
Q8.	Was appropriate statistical analysis used?
Case-control studies (JBI checklist):	
Q1.	Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?
Q2.	Were cases and controls matched appropriately?
Q3.	Were the same criteria used for identification of cases and controls?
Q4.	Was exposure measured in a standard, valid and reliable way?
Q5.	Was exposure measured in the same way for cases and controls?
Q6.	Were confounding factors identified?
Q7.	Were strategies to deal with confounding factors stated?
Q8.	Were outcomes assessed in a standard, valid and reliable way for cases and controls?
Q9.	Was the exposure period of interest long enough to be meaningful?
Q10.	Was appropriate statistical analysis used?