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**Dental Implant Material Related
Molecular Changes in Peri-implantitis –
A Systematic Review and Integrative
Analysis of Omics Studies**

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For my lovely family

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Abstract

BACKGROUND: Implant wear debris is increasingly being considered in dental research to induce proinflammatory reactions and thus acts as a key factor in the destructive processes of peri-implantitis.

This thesis provides a systematic review of the recent literature addressing the issue of titanium particles affecting the mucosal tissue surrounding dental implants by altering the molecular signatures (e.g., transcriptome, proteome, epigenome, genome) and therefore promote peri-implantitis.

METHODS: Using three literature databases (Medline, Embase, Cochrane), we conducted a systematic and computer-assisted search for eligible contributions based on *a priori* defined PICO – ‘omics’-studies investigating titanium exposure in cell cultures or comparing molecular signatures in healthy and infected peri-implant sites and/or healthy and periodontitis affected teeth in animals/humans. After risk of bias assessments, lists of significant differentially expressed genes (DEG) and proteins as well as results of Gene Ontology and pathway enrichment analyses were generated to find common patterns. The significance of the overlap of genes in multiple studies was estimated by Monte Carlo simulations and their ranking was verified using Robust Rank Aggregation. Unpublished data of a large-scale transcriptome study comparing peri-implantitis and periodontitis was used to validate the results of the systematic review.

RESULTS: Out of 4291 screened articles we found 21 eligible publications. In four studies, a significant overlap of gene expression was found in oral-related cells when exposed to titanium particles, highlighting potentially relevant DEG associated with titanium exposure. Moreover, changes in biological processes such as immune/inflammatory response, stress response, cell cycle arrest/apoptosis, and metabolism were related to the presence of titanium in both transcriptomic and proteomic analysis. Epigenetic changes assessed by global DNA hypomethylation induced by titanium exposure were measured but not consistent. Distinct gene expression patterns were evident in both healthy and inflamed peri-implant tissues in comparison to healthy/diseased periodontal tissues in animal or human studies. However, the significance of the findings was limited by the varying experimental designs, the small sample sizes and the lack of availability of published data in the included articles of the systematic review.

The verification of the results of the systematic review on basis of the functional enrichment analyses substantiated the assumption of an enhanced inflammatory host response triggered by the implant material in peri-implantitis tissue.

CONCLUSION: The consistent transcriptome and proteome alterations caused by titanium exposure in various cell lines support the hypothesis that the observed different molecular patterns between peri-implantitis and periodontitis are also linked to titanium wear debris. Amplifying the harmful impacts of an excessive immune response stimulated by the toll-like receptor signaling pathway and MAPK signaling pathway could be one potential mechanism.

Zusammenfassung

HINTERGRUND: In der zahnmedizinischen Forschung wird zunehmend davon ausgegangen, dass Implantatabrieb proinflammatorische Effekte auslöst und daher eine Schlüsselrolle bei den destruktiven Prozessen der Periimplantitis spielt.

Diese Arbeit bietet einen systematischen Überblick über die aktuelle Literatur, die sich mit der Frage beschäftigt, ob Titanpartikel das Schleimhautgewebe um Zahnimplantate beeinflussen, indem sie die molekularen Signaturen (z. B. Transkriptom, Proteom, Epigenom, Genom) verändern und dadurch Periimplantitis fördern.

METHODEN: Mit Hilfe von drei Literaturdatenbanken (Medline, Embase, Cochrane) wurde eine systematische und computergestützte Suche nach geeigneten Beiträgen auf der Grundlage von zuvor definierten PICOs durchgeführt – “omics”-Studien, die die Titanexposition in Zellkulturen untersuchen oder molekulare Signaturen in gesundem und infiziertem periimplantärem Gewebe und/oder gesunden und von Parodontitis betroffenen Zähnen bei Tieren/Menschen vergleichen. Nach einer Bewertung des Verzerrungsrisikos der Studien wurden Listen signifikant unterschiedlich exprimierter Gene (DEG), Proteine und Ergebnisse von “Gene-Ontology”- und “Pathway”-Anreicherungsanalysen zusammengestellt, um gemeinsame Muster zu erkennen. Die Signifikanz von sich überschneidenden Genen in mehreren Studien wurde mittels Monte-Carlo-Simulationen bewertet, und ihre Rangfolge wurde mittels robuster Rangaggregation überprüft. Unveröffentlichte Daten einer groß angelegten Transkriptomstudie zum Vergleich von Periimplantitis und Parodontitis wurden zur Validierung der Ergebnisse der systematischen Überprüfung herangezogen.

ERGEBNISSE: Von den 4291 geprüften Artikeln waren 21 Veröffentlichungen für die systematische Übersichtsarbeit geeignet. In vier Studien wurde eine signifikante Überlappung der Genexpression in Titan-exponierten Zellen im Mundraum gefunden, wodurch potenziell relevante DEG im Zusammenhang mit einer Titanexposition aufgezeigt wurden. Darüber hinaus wurden Veränderungen in biologischen Prozessen wie Immun-/Entzündungsreaktion, Stressreaktion, Apoptose und Stoffwechsel sowohl in Transkriptom- als auch in Proteomanalysen mit Titan in Verbindung gebracht. Titanbedingte epigenetische Veränderungen (globale DNA-Hypomethylierung) wurden festgestellt, waren jedoch uneinheitlich. Sowohl gesundes als auch entzündetes periimplantäres Gewebe zeigte im Vergleich zu gesundem/krankem parodontalen Gewebe in Tier- oder Humanstudien unterschiedliche Genexpressionsmuster. Die Aussagekraft

der Ergebnisse wurde jedoch durch die unterschiedlichen Versuchsaufbauten, die geringen Stichprobenumfänge und die mangelnde Verfügbarkeit veröffentlichter Daten in den Artikeln der systematischen Übersichtsarbeit eingeschränkt.

Die Überprüfung der Ergebnisse der systematischen Übersichtsarbeit auf der Grundlage der funktionellen Anreicherungsanalysen bekräftigte die Annahme einer durch das Implantatmaterial induzierten verstärkten entzündlichen Wirtsantwort im Periimplantitisgewebe.

SCHLUSSFOLGERUNG: Gemeinsame Transkriptom- und Proteomveränderungen aufgrund von Titanexposition in verschiedenen Zelltypen unterstützen die Hypothese, dass die beobachteten unterschiedlichen molekularen Muster zwischen Periimplantitis und Parodontitis auch mit Titanabrieb zusammenhängen. Ein möglicher Mechanismus ist die Verstärkung der nachteiligen Auswirkungen einer überschießenden Immunantwort, die durch den Toll-like-Rezeptor-Signalweg und den MAPK-Signalweg induziert wird.

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List of Abbreviations

| | | |
|------------------------------------|-----------|--|
| 5hmC | | 5-hydroxymethylcytosine. |
| 5mC | | 5-methylcytosin. |
| Ag | | Silver. |
| AK | | Annika Kröger (reviewers' name). |
| Cd | | Cadmium. |
| ceRNA | | Competing endogenous RNA. |
| CI | | Confidence interval. |
| DAVID | | Database for annotation, visualization and integrated discovery. |
| DE | | Differentially expressed. |
| DEG | | Differentially expressed genes. |
| DEP | | Differentially expressed proteins. |
| ECM | | Extracellular matrix. |
| EU | | European Union. |
| FC | | Fold change. |
| FCS | | Functional class scoring. |
| FDR | | False discovery rate. |
| Fe₂O₃ | | Ferric oxide. |
| GO | | Gene Ontology. |
| GSEA | | Gene set enrichment analysis. |
| GW | | Gregor Würfl (reviewers' name). |
| KEGG | | Kyoto Encyclopedia of Genes and Genomes. |
| LC-MS/MS | | Liquid chromatography with tandem mass spectrometry. |
| LF | | Lena Freitag (reviewers' name). |
| lncRNA | | Long non-coding RNA. |
| LPS | | Lipopolysaccharide. |

List of Abbreviations

| | | |
|------------------------|-----------|--|
| MAQC | | MicroArray Quality Control. |
| miRNA | | Micro RNA. |
| MK | | Moritz Kebschull (reviewers' name). |
| MMP | | Matrix metalloproteinase. |
| NA | | Not applicable. |
| NOS | | Newcastle-Ottawa Scale. |
| NP | | Nanoparticles. |
| ORA | | Over-representing analysis. |
| PAMPs | | Pathogen-associated molecular patterns. |
| PCR | | Polymerase chain reactions. |
| PICO/PECO | | Research questions according to the PICO method (population, intervention/exposure, control, outcome). |
| ROS | | Reactive oxidative species. |
| RRA | | Robust Rank Aggregation. |
| RT-PCR | | Real-time PCR. |
| SOD | | Superoxide dismutase. |
| Ti | | Titanium. |
| TiO₂ | | Titanium dioxide. |
| TLR | | Toll-like receptor. |
| TS | | Thomas Spinell (reviewers' name). |
| SiO₂ | | Silicon dioxide. |
| ZnO | | Zinc oxide. |

Introduction

Peri-implantitis is a challenging and widespread complication affecting dental implants; this disease, characterized by chronic inflammation and subsequent bone loss around dental implant sites, is a leading cause of implant failure [1–5]. There are several risk factors for peri-implantitis related to the patient, the environment or the practitioner, with some being similar to those of periodontitis [6]. For example, a university-representative cross-sectional study – including 99 patients and 458 implants with a prevalence of peri-implantitis exceeding 30% – identified smoking, the presence of plaque and implant malposition as risk factors, as well as interapproximal flossing/brushing and medication with anticoagulants as protective factors for peri-implantitis [7]. In addition, peri-implantitis tends to occur more often in patients with a history of periodontitis which has also been shown in several systematic reviews [7–13]. This and the fact that peri-implantitis has a clinical phenotype similar to that of periodontitis, including dysbiotic microbiota [14], indicates a similar pathomechanism of the two gum diseases. However, there are significant differences in disease progression rates between the two entities, indicating unique pathophysiological mechanisms in each [15–17]. Thus, untreated peri-implantitis appears to progress faster and in a non-linear, accelerating pattern in contrast to periodontitis [18]. Histopathological differences were also evident in several animal and human studies [14,19–21]. In an animal study on dogs, Carucac et al. noted a higher prevalence of neutrophils and osteoclasts in tissues affected by peri-implantitis than in periodontitis samples. Furthermore, it was demonstrated that peri-implant lesions, unlike periodontal lesions, are larger, less encapsulated and thereby more extended to the crestal bone [22]. Therefore, the reasons for these histopathological dissimilarities between the two diseases can be

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found in the absence of root cementum, periodontal ligament and supra-crestal attachment fibers on implants and are consistent with the faster progression rate of peri-implantitis [23]. Further explanations for the discrepancies of peri-implantitis and periodontitis are present in the literature, but the underlying mechanisms remain unclear. For example, structural differences such as reduced vascularity and fibroblast-to-collagen ratios may cause the increased extension of infiltrating inflammatory cells in peri-implant compared to dentogingival tissue [14,24]. In addition, the clear disparity between an implant and a natural tooth – the material itself – should be taken into account and thus how implant material-related factors influence the onset and progression of peri-implantitis [17].

Besides the surface roughness [25], the material composition, specifically titanium, seems to influence peri-implant lesions. In the field of orthopedics, the leading reason of implant revision is aseptic loosening and probably the best studied theory is the activation of a localized inflammatory reaction and bone resorption mediated by osteoclasts due to the presence of wear debris particles [17,26,27]. Since the oral cavity is a dynamic, corrosive environment, several factors can lead to bio-tribocorrosion on titanium dental implants, which three recent reviews discuss extensively. [28–30]. Tribocorrosion results in simultaneous degradation of implant surfaces by wear (mechanical, leading to particle release) and corrosion (electrochemical, mostly leading to ion release) processes [31]. Mechanical factors producing wear particles include micro-movements during mastication, overloading, but also treatment of peri-implantitis [28]. The ability of titanium implants to resist corrosion is based on the titanium oxide layer [30]. It can be altered by a decrease in pH (as a consequence of microbial metabolic products or inflammation) or fluoride ions by forming the corrosive hydrofluoric acid (HF) resulting in titanium release into the surrounding tissue [29]. This has been shown in various studies [30,32–35]. Additionally, in a recent systematic review, titanium as well as other metallic particles – with variations in shape and size (100 nm to 54 μm) – were found to be detectable in both soft (epithelial cells, connective tissue, and inflammatory cells) and hard (bone crest and bone marrow) tissues surrounding dental implants; the

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concentration of these particles is notably higher in peri-implant lesions compared to healthy sites. [17,36]. Titanium is no longer considered completely bioinert [37]. This is also reflected in the EU's recent decision to ban titanium dioxide as a food additive (E171) by 2022 based on the updated safety assessment of the European Food Safety Authority (EFSA) as genotoxicity of enriched TiO₂ particles in the organism cannot be ruled out [38]. The effect of titanium on tissue is well described in the literature with regard to certain aspects, such as the pro-inflammatory effect due to elevated cytokine levels (like TNF- α , IL-1 β , and IL-6 cytokines) [39–42] or modification in bone metabolism through an altered RANKL/osteoprotegerin ratio [43].

In order to obtain a holistic understanding of cellular processes at multiple levels, high-throughput technologies are gaining in importance, providing insights into health and disease [17,44–46]. Hence, ‘omics’ research has developed strongly in recent years [47]. Omics data types can be Genomics (searching for genetic variants linked to diseases or response to treatment), Epigenomics (identifying reversible, genome-wide modifications of DNA or proteins associated with DNA), Transcriptomics (qualitative and quantitative genome-wide analysis of RNA levels), Proteomics (quantification of peptide abundance, post-translational modification, and protein interaction), Metabolomics (quantifying simultaneously multiple products of metabolic processes in cells) or Microbiomics (investigating all microorganisms of a certain community) [48]. In dentistry, especially the latter two methods have been widely used in caries and periodontology research supporting the viewpoint of dysbiosis of supra-/subgingival biofilms [49,50].

As peri-implantitis is a complex and multifactorial disease [15], omics technologies are useful to gain a holistic perspective on the underlying cellular processes. Whilst the effects of titanium on specific biomarkers as well as clinical and histological differences between peri-implantitis and periodontitis have been thoroughly discussed in the existing literature, there remains a lack in systematically reviewing the results of omics approaches on this subject.

Hence, a systematic review of current omics research was carried out to examine the cellular effects of titanium at a global molecular level [17]. Furthermore, it

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was investigated whether this material-related impact of titanium dental implants alters the mucosal chronic inflammatory response leading “to differences between the diseases peri-implantitis and periodontitis at the transcriptome, proteome, genome or epigenome level” [17,51]. Based on pre-registered protocols, three major literature databases were, therefore, double screened for relevant articles. “To identify common patterns between comparable studies, results were integrated [on the basis of] differentially expressed genes/proteins and functional enrichment analysis” [17]. “The significance of overlapping genes across multiple studies was assessed via Monte Carlo simulations and their ranking was verified using Robust Rank Aggregation” [17].

In order to cross-check the results from the systematic review, these were compared with data from a large-scale transcriptome study with a well-phenotyped cohort of peri-implantitis and periodontitis patients.

This doctoral thesis is aimed to enhance understanding of the pathophysiology of peri-implantitis, reveal research gaps, and identify promising fields of research for improved dental implant success.

1

Methods

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1.1 Systematic Review

1.1.1 Registration and Protocol

The systematic review was pre-registered at PROSPERO (*in vitro* and human studies: CRD42021252402; animal studies: CRD42021252692) and designed in accordance with the PRISMA guidelines [17,51,52].

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1.1.2 Focused Research Questions

The research queries below were structured following the PICO scheme [53]. These are based on the study populations used – cell cultures (PECO 1), animals (PICO 2) and humans (PICO 3) – to investigate whether titanium dental implants affect surrounding tissue by altering molecular signatures including the transcriptome, proteome, genome or epigenome. The abbreviation ‘P’ for population, ‘E’ for exposure or ‘I’ (intervention), ‘C’ for control group and additionally ‘T’ for time were used. An overview of test and control group of respective PICO is given in Table 1.

PECO 1 [17]:

- a) *“In human cell cultures [P], how do molecular signatures [O] change upon exposure to titanium particles [E] compared to no exposure [C]?”*
- b) *“In human cell cultures [P], how do molecular signatures [O] change upon exposure to solutions with titanium ions [E] compared to solutions without titanium ions [C]?”*
- c) *“In human cell cultures [P], how do molecular signatures [O] change upon exposure to titanium particles/solution [E] compared to other metallic particles/solutions [C]?”*
- d) *“In human cell cultures [P], how do molecular signatures [O] change upon exposure to titanium particles/solution associated with oral bacteria or its pathogen-associated molecular patterns (e.g., LPS) [E] or without association with oral bacteria [C]?”*

PICO 2 [51]:

- a) *“In animals [P], how do molecular signatures [O] differ between healthy dental implants [I] compared to healthy teeth [C]?”*
- b) *“In animals [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [I] compared to dental implants without an inflammation [C]?”*

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- c) “In animals [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [I] and around periodontally compromised teeth [C]?”

PICO 3 [51]:

- a) “In systemically healthy, non-smoking humans [P], how do molecular signatures [O] differ between healthy dental implants [I] compared to healthy teeth [C] a minimum of one year after implant placement [T]?”
- b) “In systematically healthy, non-smoking humans [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [I] compared to dental implants without an inflammation [C] a minimum of one year after implant placement [T]?”
- c) “In systemically healthy, non-smoking humans [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [I] and around periodontally compromised teeth [C] a minimum of one year after implant placement [T]?”

Table 1.1: Overview of test and control groups per PICO

| PICO | Intervention/Exposure | Control |
|--------|---|--------------------------------|
| 1a | Titanium particles | No titanium particles |
| 1b | Solutions with titanium ions | Solution without titanium ions |
| 1c | Titanium particles/ions | Other metallic particles/ions |
| 1d | Titanium particles/ions associated with oral bacteria or its pathogen-associated molecular patterns (e.g., LPS) | Titanium particles/ions |
| 2a, 3a | Healthy dental implant | Healthy tooth |
| 2b, 3b | Dental implant with peri-implantitis | Healthy implant |
| 2c, 3c | Dental implant with peri-implantitis | Tooth with periodontitis |

1. Methods

1.1.3 Inclusion and Exclusion Criteria

In general, studies without any limitations on publication date were included, but they had to be published in either English or German [17,51]. Moreover, eligible studies were required to use appropriate methods to obtain omics data, such as microarray analyses or mass spectrometry-based proteomics; measurement of single biomarkers, for example, was not sufficient. Case reports or case series were excluded because of their non-comparative design.

In vitro studies were included that utilized human cell lines equivalent to a peri-implantitis model – which means cells associated with oral tissues or the immune system [17]. To prevent the study selection from being overly restrictive, the use of pure titanium particles or ions was acceptable, regardless of their size and concentration [17].

Only implants made of titanium were considered in the *in vivo* studies. All animal studies with a study design according to the PICO questions were included, unless tissue samples unrelated to an implant or tooth or animals with comorbidities were involved [51].

For human studies, it was a prerequisite that the tissue samples were collected at least one year after insertion of the titanium dental implants, and the study population had to consist of adults (≥ 18 years) who were both systemically healthy and non-smokers[51]. For genomic assays, blood samples were also considered acceptable [51].

1.1.4 Information Sources and Search Strategy

The initial search was performed on 07/01/2021 and refreshed preceding the definitive analysis [17,51] to include the most recent eligible articles (01/27/2022). Three distinct electronic databases were used for the literature search [17,51]:

- MEDLINE (Medical Literature Analysis and Retrieval System Online via PubMed) ¹

¹<https://pubmed.ncbi.nlm.nih.gov/>

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- Embase ²
- Cochrane Library ³

In addition, the subsequent search strategies were applied to manually find potentially relevant articles: Related reference lists of all potential full texts were searched [51]; the ‘Connected Papers’ website ⁴ was utilized to identify analogous studies based on significant overlaps in citations and references [17,51]. Moreover, five journals were screened from January 2001 to July 2021 via the specified websites:

- Journal of Periodontology ⁵
- Journal of Dental Research ⁶
- Journal of Clinical Periodontology ⁷
- Clinical oral Implants Research ⁸
- OMICS: A Journal of Integrative Biology ⁹

The complete search strategy with a full list of search terms is available in Appendix B. Following a naive search to obtain a sample of eligible studies, the R package `litsearchr` (version 1.0.0) was applied to find more relevant terms through text mining and keyword co-occurrence networks [17,54]. This Boolean search strategy was customized for each specific database. For instance, MeSH terms and Emtree terms received special labeling [17,51]. A language restriction to English and German and a search restriction to titles and abstracts were applied as filters.

1.1.5 Study Selection and Data Collection Process

After the removal of duplicates, titles and abstracts resulting from the search strategy described in Appendix B were reviewed independently by two assessors

²<https://www.embase.com/>

³<https://www.cochranelibrary.com/>

⁴<https://www.connectedpapers.com/>

⁵<https://aap.onlinelibrary.wiley.com/journal/19433670>

⁶<https://journals.sagepub.com/home/jdr>

⁷<https://onlinelibrary.wiley.com/journal/1600051x>

⁸<https://onlinelibrary.wiley.com/journal/16000501>

⁹<https://home.liebertpub.com/publications/omics-a-journal-of-integrative-biology/>

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(LF, TS) [17,51]. To verify the agreement between both reviewers, a preliminary calibration exercise was carried out. Subsequently, the full texts of the articles found previously were retrieved, if both evaluators concurred that they potentially met all inclusion criteria and had relevance. Finally, the eligibility of full texts were reviewed separately by two authors (LF, TS) [17,51]. In addition, Cohen's kappa was utilized to calculate inter-rater reliability [55] using the R package `irr` (version 0.84.1) [17]. The screening process was conducted with the R package `revtools` (version 0.4.1) [56] to simplify the importing of data and de-duplication [17,51]. Generally, any discrepancies in decisions on inclusion or exclusion of texts were resolved after consulting with a third reviewer (AK) [17,51].

Two reviewers (LF, AK) independently performed duplicate data extraction from both text and graphs via a pre-established and validated spreadsheet [17,51]. As recommended by the Cochrane Handbook, the implementation and comparison of the data extraction of the two authors was facilitated with the R program and Excel [53]. Additionally, incomplete or missing data were requested from the authors of the corresponding studies via email [17]. In case of multiple papers reporting on the same experiment, only the relevant data from one study were extracted. Again, potential discrepancies in decisions were resolved after consulting a third reviewer (AK) [17].

Depending on the respective PICO question, the following variables were searched for in the selected studies: author, year of publication, test and control group, sample size, methods, exposure time, cell line, animal model and population/implant/titanium particles characteristics. In addition, "published Gene Ontology (GO) terms and pathways of functional enrichment analyses [were extracted from] each study" [17,51]. Whenever obtainable, "cutoff criteria and lists of statistically significant differentially expressed genes/proteins" (DEG/DEP) were compiled [17,51]. "If raw data was deposited in the GEO database, it was analyzed via the GEO2R web application to [generate] DEG lists with fold change (FC) greater than or equal to 1.5 ($\log_2FC \geq |0.58|$) and adjusted p-value < 0.05 ([using the] Benjamini-Hochberg method)" [17,57]. Respective test and control gene expression files as well as the provided annotation files were loaded with default settings via

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this interface to R and differential expressed gene analysis was performed using the `limma` package [57]. Furthermore, gene symbols were uniformed following the HUGO Gene Nomenclature (using multi-symbol-checker provided by HGNC ¹⁰) [17].

1.1.6 Data Analysis Methods

To integrate the results – lists of DE genes or proteins – from each study, two different methods were used and are subsequently compared with each other.

First, a vote-counting strategy similarly used in other systematic reviews to rank mRNAs or microRNAs (miRNAs) was applied [17,58–61]. The ranking of DE genes and proteins that were equally expressed in multiple studies was based on the number of studies or experiments (within studies) in which they were reported, followed by average fold change [17]. Subsequently, the significance in the overlaps found in gene or protein expression was evaluated using Monte Carlo simulations as described previously [17,58,59,62]. For transcriptome studies, for example, total gene lists with ENTREZ IDs of the microarray platforms used were acquired from the corresponding websites [17]. “In case of RNA sequencing analysis the [entire] human genome was [utilized to generate] a random sample of overlapping genes” [17]. Thereby, a random selection with the same number of upregulated and downregulated genes as in the respective studies was made out of the total gene lists and the overlap was calculated in the same manner as for real data [17]. “These steps were [iterated] 10,000 times” [17]. Finally, “the p-value was estimated by dividing the number of simulations in which the permuted overlap was greater than or equal to the reported overlap (NGE), by the number of simulations (n): $p = \frac{NGE+1}{n+1}$ ” [17].

For validation of these results, a second approach – robust rank aggregation (RRA) – was employed [17]. This method relies “on ordered statistics and [is] implemented in the R package `RobustRankAggreg` (version 1.1)”, facilitating the identification of significant elements between different DEG lists [17,63]. For this purpose, the lists of DE genes and proteins from the selected studies were ranked according to the logarithmic fold change values in descending order [17]; so, for

¹⁰<https://www.genenames.org/tools/multi-symbol-checker/>

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example, the gene whose expression values differed most between test and control groups is at the top of the list. The algorithm checks the position of each gene or protein in the ranking lists and compares it to the case where all lists are ordered randomly, thus generating p-values for every gene or protein [17,63]. Small p-values imply that the observed ranking would be highly improbable if random ordering were assumed [17,63].

If adequate data was provided, the web-based bioinformatics resources of DAVID¹¹ (Database for Annotation, Visualization and Integrated Discovery, version: DAVID 2021 (Dec. 2021)) were used to detect enriched gene ontology and pathways in genes showing equal expression in at least two experiments [17]. Herein, the functional annotation chart tool was used based on modified Fischer's exact test with default settings. Enriched GO terms/pathways were ordered by p-values corrected by the false discovery rate (FDR).

In order to compare the published results of functional enrichment analyses of each study, the GO terms and pathways were categorized based on their 'parent term' identified via the website 'QuickGO'¹² or broader terms in the hierarchy available in the Kyoto Encyclopedia of Genes and Genomes (KEGG)¹³ or Reactome pathway database¹⁴, respectively. The biological processes most commonly altered in several studies are presented in bar charts.

All analyses were conducted using the software environment R (version 4.1.1). R scripts are added in the Supplemental Materials.

1.1.7 Study Risk of Bias Assessment

Two reviewers individually evaluated the risk of bias of the included articles (LF, GW) [17,51]. Any disagreements between the reviewers were resolved consensually after consulting a third reviewer (AK) [17,51].

¹¹<https://david.ncifcrf.gov/tools.jsp>

¹²<https://www.ebi.ac.uk/QuickGO/annotations>

¹³<https://www.genome.jp/kegg/pathway.html>

¹⁴<https://reactome.org/>

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The Newcastle-Ottawa Scale (NOS) for cross-sectional studies modified by Modesti et al. was applied to assess the quality of human studies [51,65]. In this tool a total of ten stars can be awarded: a maximum of five stars for the selection of a representative and sufficiently sized sample and adequate measurement approaches, two stars for the comparability of the study groups, and three stars for the appropriate ascertainment of outcomes. The domain of ‘assessment of the outcome’ was adapted so that two stars can be given if data was obtained directly from samples in the laboratory or downloaded from an official database. The non-respondent category was not applicable due to the study design with single sample collection. Studies receiving less than five stars overall are considered to have a high risk, studies with a maximum of seven or ten stars achieve a medium/low risk of bias in total.

The SYRCLE’s risk of bias tool was applied to the included animal studies [51,66]. Potential sources of bias were appraised using a series of questions about selection, performance, discovery, abandonment, or selective reporting. These questions were answered with ‘yes’, ‘no’ or ‘unclear’ reflecting low, high or inadequately assessable risk of bias.

Since there are no standardized tools for evaluating the risk of bias in *in vitro* studies, the OHAT Risk of Bias Tool for Human and Animal Studies [67] was adapted to *in vitro* studies, as other reviewers have already successfully used this approach in their systematic reviews [17,68–70]. The response options consisted of ‘definitely low’, ‘probably low’, ‘definitely high’, ‘probably high’ and ‘unclear’. Following key categories representing the highest effect on the overall bias were utilized: “Identical experimental conditions between study groups, complete outcome data, selective reporting and adequate sample size” [17]. Subsequently, the studies were classified in three tiers:

- Tier 1: If a study was scored ‘definitely low’ or ‘probably low’ in all key categories AND ‘definitely low’ or ‘probably low’ in at least half of the other RoB domains

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- Tier 2: If a study did not fulfill the criteria for Tier 1 or Tier 3
- Tier 3: If a study was scored ‘definitely high’ or ‘probably high’ in all key categories AND ‘definitely high’ or ‘probably high’ in at least half of the other RoB domains

The visualization of the results of the risk of bias assessment was performed with the R package `robvis` (version 0.3.0.900) [71].

1.2 Comparison of a Large-Scale Transcriptome Study and Results of the Systematic Review

In order to further probe the results of the systematic review, a DEG list obtained from RNA sequencing analysis of peri-implantitis and periodontitis specimens was used (n=45/condition, matched for maximum probing depth: peri-implantitis 8.07 ± 1.95 mm, periodontitis 8.09 ± 1.61 mm) [72]. The unpublished data was kindly provided by Moritz Kepschull¹⁵.

Sixty-six systemically healthy, non-smoking, fully clinically phenotyped patients from five university dental clinics participated in this cross-sectional study. Inclusion criteria similar to those used in the systematic review were applied (see section 1.1.3). In particular, tissue around the implant was harvested if there was a probing depth of at least 5 mm, bleeding on probing, and radiographic bone loss of at least 3 mm. The interdental papilla was used as tissue sample in the periodontitis group (probing depth of at least 5 mm, bleeding on probing, clinical attachment loss of at least 4 mm) [72].

After total RNA isolation from the 90 biopsies and its quality control, the mRNA was transcribed into cDNA for further RNA sequencing (HiSeq2500 sequencing system, Illumina). The quality of the raw data of 4.9 billion sequencing reads was checked using the software FastQC. Subsequently, the sequences were aligned to the human genome (STAR Aligner) and the genes were quantified (FeatureCounts)

¹⁵Professor Moritz Kepschull, Chair of Restorative Dentistry, School of Dentistry, Institute of Clinical Sciences, University of Birmingham, Birmingham, UK

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as well as their numbers normalized. Differential gene expression analysis was performed via *limma* / *voom*. Thereby, a mixed regression model was applied instead of a *t*-test, controlling for gender and number of samples per patient. Multiple testing was taken into account via controlling the false discovery rate (FDR) according to Benjamini-Hochberg [72].

1.2.1 Comparative Analysis Based on Functional Enrichment Analysis

To compare the results of the systematic review regarding transcriptomic differences between peri-implantitis and periodontitis with the large-scale study described previously (see section 1.2) GO and pathway enrichment analysis were performed on the DEG of this study. Thereby, enrichment analysis based on a modified Fisher’s exact test of DAVID bioinformatics resources (see section 1.1.6) were utilized to identify gene ontology terms (biological processes) and pathways that were most affected by genes differentially expressed between peri-implantitis and periodontitis [64]. ENTREZ IDs of DEG with fold change ≥ 1.5 and adj. p-value < 0.05 were loaded into the data-mining environment of DAVID and the whole human genome was applied as background genes for the further analyses. In particular, the functional annotation clustering tool was used by grouping GO terms that have many genes in common based on co-occurrence measured via *kappa* statistics to reduce redundant terms with similar biological processes. This results in broader ‘biological modules’ that are easier to interpret [73]. Herein, pathway (KEGG_PATHWAY) and GO (GOTERM_BP_DIRECT) enrichment results were implemented in the functional annotation clustering tool with default settings.

1.2.1.1 ClusterProfiler

Using the ‘compareCluster’ function of the *clusterProfiler* R package (version: 4.2.2), functional enrichment results based on over-representing analysis applying a Fisher’s exact test for multiple gene lists (all DEG with FC ≥ 1.5 and p-value < 0.05) were compared [74]. The visualization was done in the form of a dot plot.

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1.2.2 Comparative Analysis at the DEG Level

The overlap of DEG with same direction of expression between the large-scale transcriptome study and studies included in the systematic review was calculated using the following simple formula, if a list of all DEG was available:

$$\textit{overlap} = \frac{\text{number of common genes}}{\text{number of all DEG of one list}}$$

For this purpose, the raw data was further analyzed for a study that was suitable for PICO question 3c in the systematic review [75]. After conducting a list of DEG via the GEO2R web application (see section 1.1.5), a subsequent annotation was required because the annotation of the platform used (GPL21287) only provided the sequence data format. Using the R package `Rsubread` (version: 2.8.2) [76], a reference genome was generated from the human protein-coding transcript sequences FASTA file downloaded from GENCODE ¹⁶ to map the sequence of each probe to a HGNC gene symbol.

¹⁶https://www.gencodegenes.org/human/release_19.html

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2.1 Systematic Review

2.1.1 Study Selection

After removing duplicates, 4291 unique articles were found via electronic searches of MEDLINE, Embase, and Cochrane Library databases. Following the initial screening phase of title and abstracts by two independent reviewers, 40 papers were selected for full text analysis. In sum, 21 articles were eligible for the subsequent

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analyses including one paper found during manual search and three during search updating (see Figure 2.1). A total of 12 relevant *in vitro* studies were identified, with some investigating multiple molecular levels, whereas only four animal and five human studies met the inclusion criteria. There were no suitable studies for PICO questions 1b, 1d, 2c, and 3a. The reasons for the exclusion of the respective articles can be checked in the Appendix A.

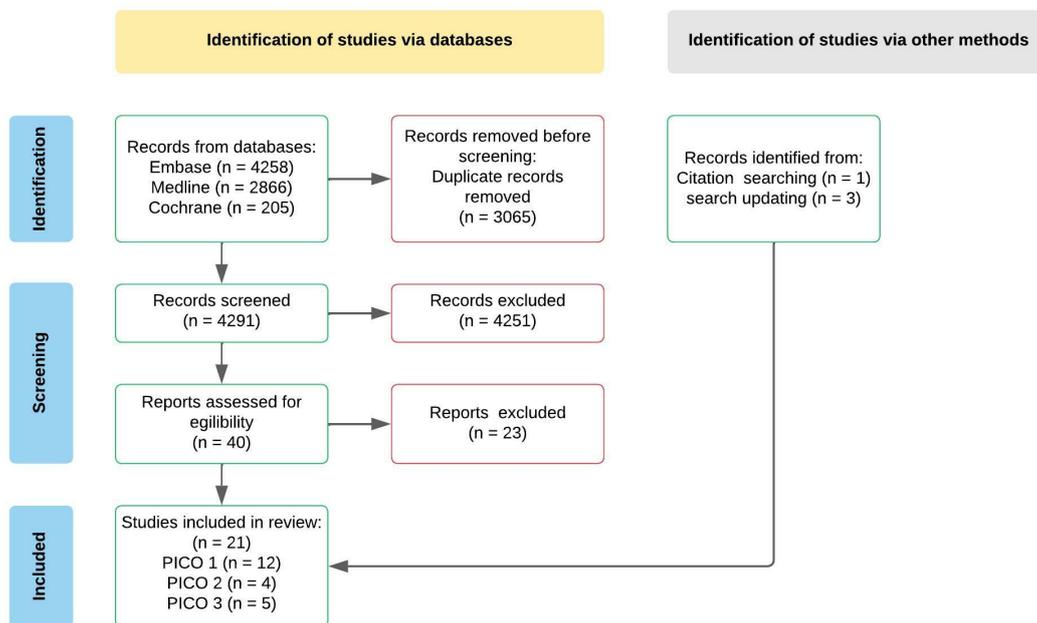


Figure 2.1: Flow diagram illustrating the screening and selection process according to the PRISMA 2020 statement, adapted from Freitag et al.,2023 with permission of the publisher [17]

2.1.2 Inter-rater Agreement

Inter-rater agreement was evaluated for the first screening stage (title and abstract) resulting in a percentage agreement of 98.9% for all screened articles. To account for the amount of agreement that would be expected by chance, Cohen’s kappa was calculated, showing a coefficient of inter-rater reliability of 0.61 (CI: 0.50 to 0.71). According to Altman, this corresponds to a good agreement between the two reviewers LF and TS [77].

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2.1.3 Study Characteristics

An overview of the study characteristics for each PICO question is presented in Tables 2.1, 2.2 and 2.3. Generally, the applied statistics and methods varied between studies [17]. For instance, the researchers conducted microarray analyses with various platforms or RNA sequence analyses and applied diverse cut-off criteria for significant DEG or DEP [17].

The *in vitro* studies showed a high level of heterogeneity with respect to the cell lines; however, most cells belong to the immune system with monocytes or macrophages representing the largest proportion (see Figure 2.2). Reported concentrations of titanium particles ranged mainly from 0.5 to 100 $\mu\text{g}/\text{ml}$ with one outlier study with a concentration of 300 $\mu\text{g}/\text{ml}$ [78]. Although three studies did not provide clear information on the concentrations used [79–81], concentrations with low to mild toxicity were consistently chosen for each cell line. All studies had short-exposure times up to 24 hours, except one study exposing cells to titanium particles for six days [82]. Furthermore, mostly titanium nanoparticles (NP) but also microparticles were tested, which differ in their crystal structure (rutile or anatase/rutile mixed forms) and particle size.

In contrast, the animal studies showed less heterogeneity in the study design. Similar to the human studies, the sample sizes were generally small, ranging from 3 to 12 specimens per group.

The study populations of the different human studies were comparable due to broadly similar inclusion criteria and definitions for periodontitis and peri-implantitis status. However, not all studies reported details regarding implant material, implant duration or pocket depths [51].

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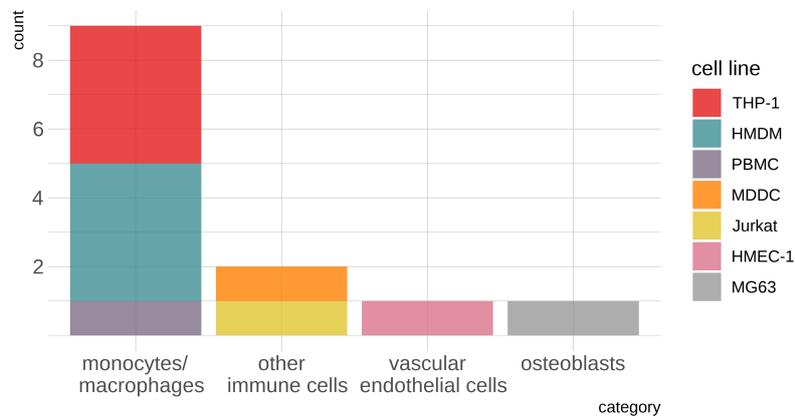


Figure 2.2: Bar plot of diverse cell lines used in studies included in the systematic review

2.1.4 Risk of Bias in Studies

Results of the risk of bias assessment of cellular, animal and human studies are outlined in figures 2.3, 2.4 and 2.5 [17,51].

All *in vitro* studies used identical experimental conditions for test and control groups as well as valid and sensitive methods to assess the outcomes. These include microarray analysis, RNA sequencing, or 2D gel-based proteomics, whereas one study with the use of gene filters was limited to the detection of a maximum of 4000 genes [81]. No information about blinding minimally impacted the overall score due to the prevalent use of automated analyses, while the absence of information about the number of replicates increased the risk of bias [17]. No study conducted power calculations and the sample sizes were at a generally low level, whereby most of the studies scored a tier of 2 in total. Because microarray analysis was performed only once in the study of Pajarinen et al. [79], the reliability of gene expression from single experiments could not be assured resulting in a higher risk of bias [17].

Blinding and randomization were not described in detail in any of the animal studies, which is less important in a split-mouth design (3 studies) than in studies with different experimental groups. Since no study provides clear information on the statistical analysis methods and the completeness of the outcome data, some concerns were raised regarding the risk of bias for these categories.

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Although the human studies had small sample sizes without prior calculation and the statistical tests were not well described in most studies, they had clear inclusion criteria, mostly similar study groups (according to sex, age, probing depth), and used appropriate measurement tools. However, the representativeness of the samples was concerning. Consequently, the overall risk of bias for human studies was considered moderate/high.

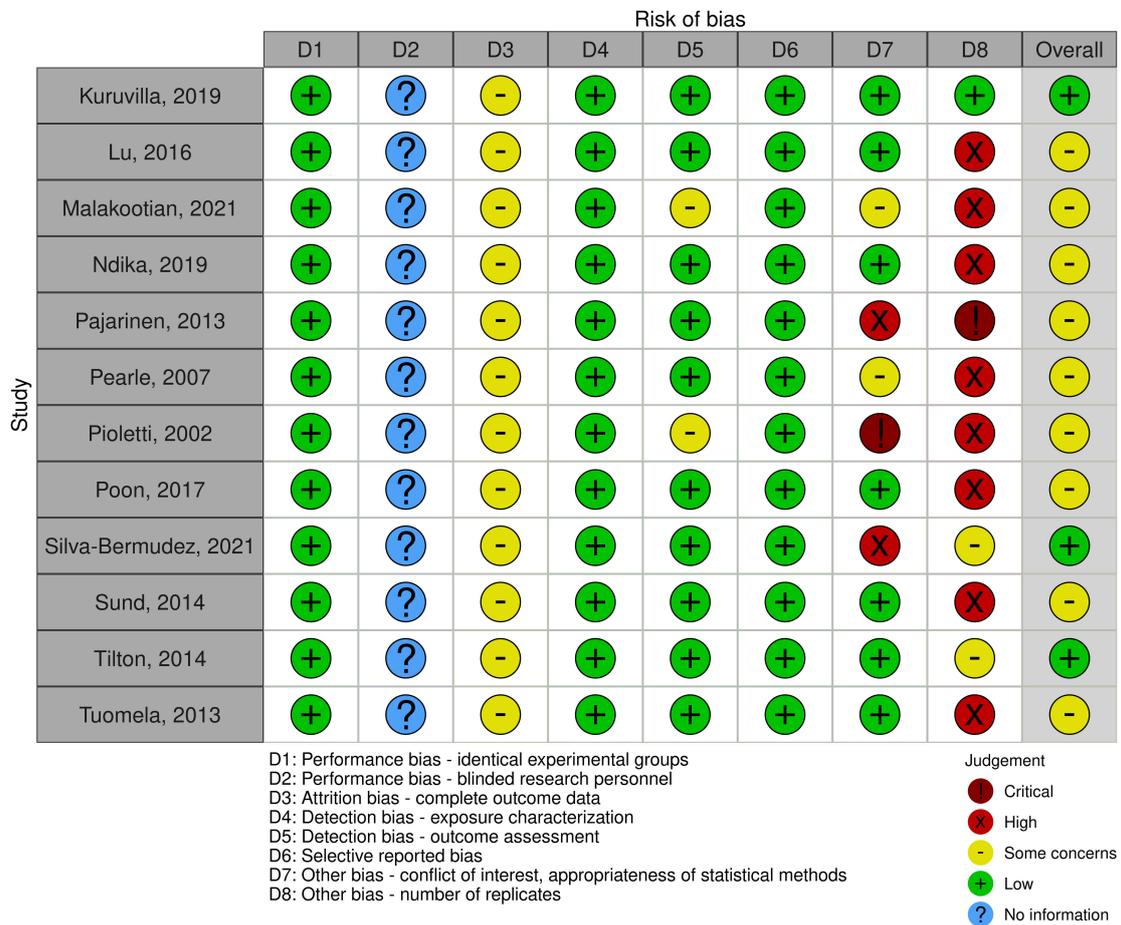


Figure 2.3: ‘Traffic light’ plot of OHAT risk of bias analyses of cell studies (PECO 1) displaying the domain-level judgment (D1-D8) for each study, adapted from Freitag et al., 2023 with permission of the publisher [17]

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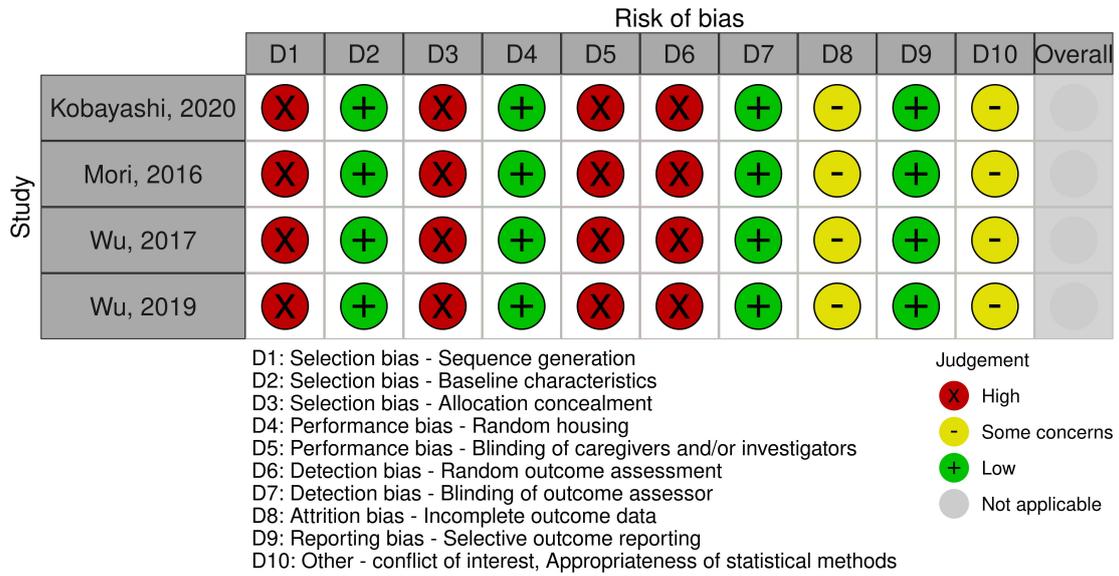


Figure 2.4: ‘Traffic light’ plot of SYRCLE’s risk of bias analyses of animal studies (PECO 2) displaying the domain-level judgment (D1-D10) for each study, adapted from Spinell et al.,2023 with permission of the publisher [51]

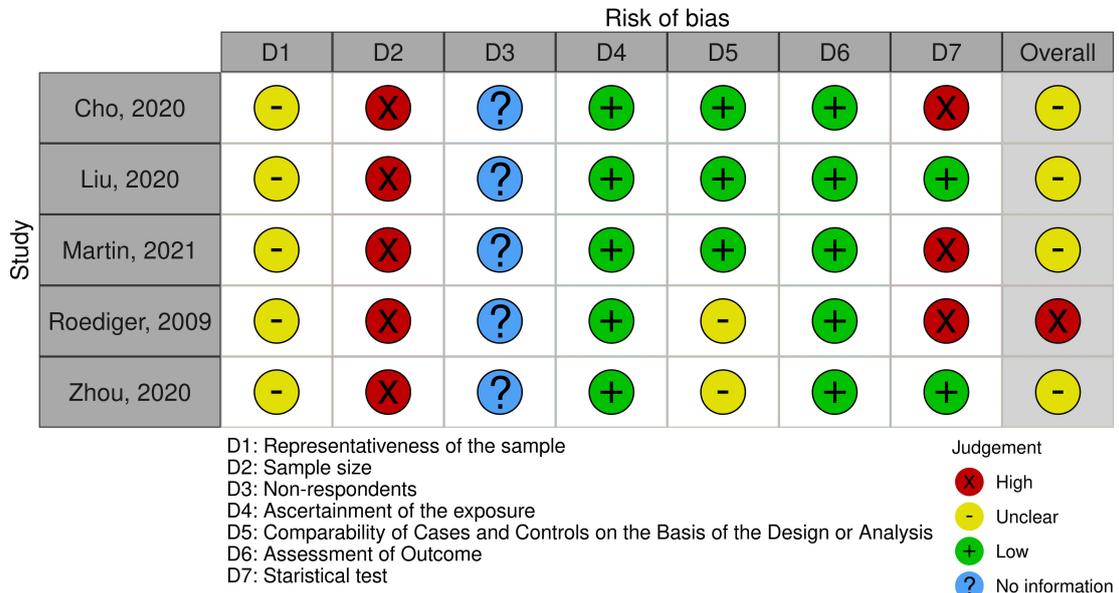


Figure 2.5: ‘Traffic light’ plot of NOS risk of bias analyses of human studies (PECO 3) displaying the domain-level judgment (D1-D7) for each study, adapted from Spinell et al.,2023 with permission of the publisher [51]

Table 2.1: Characteristics of studies eligible for PECO 1 (cell culture studies), adapted from Freitag et al., 2023 with permission of the publisher [17]

| Author, year | PECO | Test group | Crystal phase | Particle size ^a | Conc. [ug/ml] | Cell line | Follow-up [h] | Sample size ^b | Molecular level | Methods | Platform | Cutoff criteria |
|----------------------|--------|------------|--------------------|----------------------------|----------------|-----------|---------------|--------------------------|-----------------|----------------------------|---|---|
| Kuruvilla, 2019 | 1a | TiO2 | Rutile | 1-3 and 30 nm | 10 | HMEC-1 | 1 and 24 | n = 3 | Transcriptome | LC-MS/MS analysis | | $\log_2FC > 2 $, FDR < 0.01 |
| | | | | n = 6 | | | | Proteome | RNA-seq | $FC \geq 1.5$, p < 0.05 | | |
| Lu, 2016 | 1a, 1c | CuO | NA | 21 nm | 0.5 and 30 | THP-1 | 24 | NA | | LC-MS/MS of 5-(h)mC levels | | |
| | | | | 58.7 nm | | | | | | LC-MS/MS of 5-(h)mC levels | | |
| Malakootian, 2021 | 1a | TiO2 | Anatase | 44 nm | 25, 50 and 100 | PBMC | | | | 5-mC DNA ELISA kit | | |
| | | | Anatase/ rutile | 30-40 nm | | | | | | 100 | | |
| Ndika, 2019 | 1a, 1c | Ag | ZnO | 20 nm | 10 | THP-1 | 6 and 24 | n = 3 | Epigenome | small RNA-seq | | FDR < 0.05 |
| | | | | | | | | | | | | |
| Pajarinen, 2013 | | | | 3.7 μ m | | HMDM | 4 | n = 1 | | | Illumina HumanHT-12 v4 Expression BeadChip | $FC \geq 1.5$ |
| Pearle, 2007 | | | | 1 to 3 μ m | | PBMC | 3 and 6 | n = 3 | | | custom cDNA array | FDR < 0.05 |
| Pioletti, 2002 | 1a | TiO2 | NA | 45 μ m | NA | MG63 | 4 and 24 | n = 2 | | | Genefilters (GF211; Research Genetics) | $FC \geq 1.5$ (4h), $FC > 2.5$ (24h) |
| | | | | Anatase/ rutile | | | | | | | | 39.7 nm and 135.6 nm |
| Poon, 2017 | 1a, 1c | Ag | ZnO | 15.6 nm and 203.6 nm | 10 | THP-1 | 6 and 24 | n = 3 | Transcriptome | Microarray analysis | SurePrint G3 Human CGH Microarray 8x60K | $FC \geq 1.5$, FDR < 0.01 |
| | | | | 20 nm | | | | | | | | |
| Silva-Bermudez, 2021 | | | Rutile | NA | 100 | | 144 (6 days) | n = 5-9 | | | Affymetrix GeneChip Human Gene 1.0 ST Array | NA |
| Sund, 2014 | 1a | TiO2 | Anatase/ rutile | 30-40 nm | 300 | HMDM | 24 | n = 4 | Proteome | 2D gel analysis and MS | | $\log_2FC \geq 1.5 $, p < 0.01 |

Table 2.1: Characteristics of studies eligible for PECO 1 (cell culture studies), adapted from Freitag et al., 2023 with permission of the publisher [17] (*continued*)

| Author, year | PECO | Test group | Crystal phase | Particle size ^a | Conc. [ug/ml] | Cell line | Follow-up [h] | Sample size ^b | Molecular level | Methods | Platform | Cutoff criteria |
|---------------|--------|------------|----------------|----------------------------|---------------|--------------------|---------------|--------------------------|-----------------|---------------------|---|----------------------|
| Tilton, 2014 | 1a | TiO2 | NA | 7 µm | 10 and 100 | THP-1 | 1 and 24 | n = 3 | Transcriptome | Microarray analysis | Affymetrix Human Genome U133A 2.0 GeneChips | |
| | | | Anatase/rutile | 31.3 nm | 1 and 10 | | 3 and 24 | n = 5 | Proteome | LC-MS/MS analysis | | |
| Tuomela, 2013 | 1a, 1c | ZnO | ZnO | 14.7 nm | 1 and 10 | HMDM, MDDC, Jurkat | 6 and 24 | n = 3 | Transcriptome | Microarray analysis | Illumina HumanHT-12 v3 Expression BeadChips | FC ≥ 1.5, FDR < 0.05 |

Note:

Abbreviation: NA: not available, NP: nanoparticles, P: microparticles, I: ion; a: anatase, r: rutile, FC: fold change, FDR: false discovery rate

^a mean value of dry particle size

^b per test/control group (cell cultures with no particle exposure)

Table 2.2: Characteristics of studies eligible for PICO 2 (animal studies), adapted from Spinell et al., 2023 with permission of the publisher [51]

| Author, year | PICO | Study design | Species | Age, sex | Test (control) | Sample type | Follow-up | Sample size (no. of implants) ^a | Molecular level | Methods |
|------------------------|------|---------------------------------------|---------------|-----------------|-------------------------------------|------------------------------------|-----------|--|-----------------|---------------------|
| Kobayashi et al., 2020 | 2a | Prospective cohort study | Rats | 5 weeks, m | Healthy implant (healthy tooth) | Connective tissue | 4 weeks | n = 3 (3) | transcriptome | microarray analysis |
| Mori et al., 2016 | 2a | Prospective cohort study; split-mouth | Rats | 4 weeks, m | Healthy implant (healthy tooth) | Peri-implant/junctional epithelium | 4 weeks | n = 10 (5) | transcriptome | microarray analysis |
| Wu et al., 2017 | 2b | Prospective cohort study; split-mouth | Labrador dogs | 18-24 months, m | Peri-implantitis (healthy implants) | Gingival tissue | 6 months | n = 12 (12) | epigenome | miRNA-seq |
| Wu et al., 2019 | 2b | Prospective cohort study; split-mouth | Labrador dogs | 12-18 months, m | Peri-implantitis (healthy implants) | Gingival tissue | 6 months | n = 12 (12) | epigenome | miRNA-seq |

^a per test/control group

Table 2.3: Characteristics of studies eligible for PICO 3 (human studies), adapted from Spinell et al., 2023 with permission of the publisher [51]

| Author, year | PICO | Test / control | Age ^a | Men:Women | Average pocket depth | Sample size (no. of implants) ^b | Molecular level | Methods |
|-----------------------|------|------------------|------------------|-----------|----------------------|--|-------------------------------------|----------------------------------|
| Cho et al., 2020 | | Peri-implantitis | 62 | 4:1 | 6.9 | n = 5 (NA) | Transcriptome, epigenome | RNA-seq; DNA methylome profiling |
| | | Periodontitis | 53 | 1:4 | 7.8 | n = 5 (NA) | | |
| Liu et al., 2020 | 3c | Peri-implantitis | 55.7 | 4:2 | 6.7 | n = 6 (NA) | Transcriptome, epigenome (lnRNA) | |
| | | Periodontitis | 47.3 | 3:3 | 8.5 | n = 6 (NA) | | |
| Martin et al., 2021 | 3b | Peri-implantitis | 61.8 | 2:2 | NA | n = 4 (4) | | microarray analysis |
| | | Healthy implants | 56.5 | 1:2 | NA | n = 3 (3) | | |
| Roediger et al., 2009 | | Peri-implantitis | NA | NA | NA | n = 16 (16) | Transcriptome | |
| | | Periodontitis | NA | NA | NA | n = 16 (16) | | |
| Zhou et al., 2020 | 3c | Peri-implantitis | NA | NA | NA | n = 5 (NA) | Transcriptome, epigenome (mi/lnRNA) | RNA-seq |
| | | Periodontitis | NA | NA | NA | n = 5 (NA) | | |

Note:

Abbreviation: NA: not available

^a median or mean age

^b per test/control group

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2.1.5 Results of Individual Studies and Synthesis

2.1.5.1 PECO 1a

“In human cell cultures [P], how do molecular signatures [O] change upon exposure to titanium particles [E] compared to no exposure [C]?” [17]

For PICO question 1a the most studies were available (8 transcriptome [79–86], 3 proteome [78,83,84] and 3 epigenome studies [87–89]), whereby the transcriptome and proteome were analyzed simultaneously in two papers [83,84].

Transcriptome Studies Across the eight studies [79–86], researchers conducted in total 34 experiments with various particle sizes, concentrations, exposure times and cell lines[17].

In three studies encompassing 19 distinct experiments, cells with exposure to TiO₂ showed no significant differences in gene expression in comparison to cells without titanium exposure [17,80,85,86]. In these studies, both titanium nanoparticles and microparticles were investigated with exposure times of up to 24 hours and low cytotoxic concentrations within different cell lines. Although no significant DEG were detected via SAM analysis of pooled data of peripheral blood mononuclear cells exposed to TiO₂ particles for three and six hours, increased gene expression of proinflammatory T helper 1 cytokines (e.g., IL2, IFN- γ , IL9, IL13, and IL22) was present [80].

Overlap of Gene Expression Based on four of five studies with a total of 15 experiments, it was possible to compile lists with all significantly DEG with fold changes ≥ 1.5 [79,82–84], resulting in 3051 distinct genes being different in titanium particle-exposed cells [17]. One study [81] could not be included in the analyses because only initially selected genes were published [17]. Out of these, 243 (8.0%) genes displayed aligned expression patterns in at least two studies, but only 18 genes were found to be common among three, and two genes in four studies: CXCL8 and BHLHE40 [17] (see Table 5, detailed table in Supplemental Material (Table S1)). However, the extent of overlap in two, three or four studies was highly significant

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($p < 0.0001$) in the Monte Carlo simulation [17]. Thereby, an average of 58.1 (95% CI 43.7 to 72.5) genes overlapped in two studies in the simulated data compared to 219 in the real data and this simulation never reached an overlap of four trials, although this was observed twice in the actual data (see Figure 2.6). Using robust rank aggregation (RRA), “the DEG from the lists of these five studies were ranked similarly, as reflected by the smaller p-values of genes reported in multiple studies and experiments than those reported in only one study/experiment” (see Figure 2.7) [17]. For example, the gene CXCL8, identified as the most prevalent across studies, was also ranked first in the list compiled using the RRA method [17].

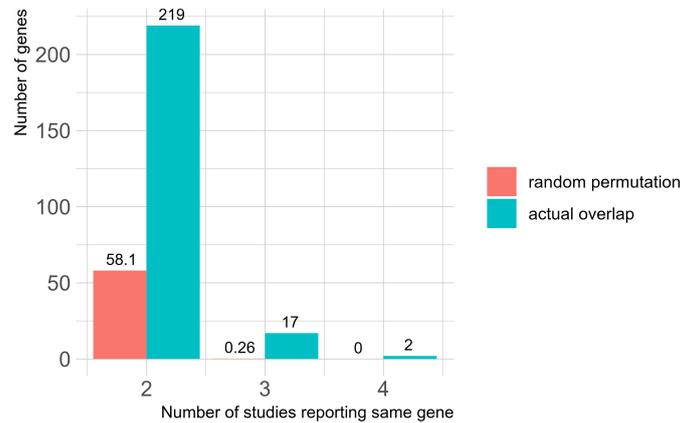


Figure 2.6: Bar plot comparing overlap analysis results of common DEG between studies (PECO 1a) by Monte Carlo simulation with observed overlap in actual data. Mean values of 10,000 permutations of simulated data are displayed.

2. Results

Table 2.4: Ranked list of common DEG across transcriptome studies of PECO 1a, adapted from Freitag et al., 2023 with permission of the publisher [17]

| HGNC symbol | Study | Experiment ^a | Score | FC.Mean | FC.Range | Direction | Reference |
|-------------|-------|-------------------------|----------|---------|----------------|-----------|------------------------|
| CXCL8 | 4 | 6 | 2.01e-12 | 1.39 | 0.74 to 2.18 | up | [79], [82], [83], [84] |
| BHLHE40 | 4 | 6 | 6.65e-10 | 1.08 | 0.76 to 1.45 | up | [79], [82], [83], [84] |
| FOSB | 3 | 5 | 7.44e-11 | 1.95 | 0.6 to 4.07 | up | [79], [83], [84] |
| CYP51A1 | 3 | 5 | 2.86e-07 | 0.84 | 0.69 to 0.99 | up | [79], [82], [83] |
| JUN | 3 | 5 | 4.36e-07 | 0.75 | 0.6 to 1.15 | up | [79], [82], [84] |
| ATF3 | 3 | 4 | 3.78e-06 | 1.33 | 1.04 to 1.86 | up | [82], [83], [84] |
| NT5DC2 | 3 | 4 | 7.38e-06 | -0.92 | -1.09 to -0.59 | down | [79], [82], [84] |
| PPP1R15A | 3 | 4 | 1.42e-05 | 0.90 | 0.68 to 1.09 | up | [79], [82], [83] |
| TBC1D2 | 3 | 4 | 2.70e-05 | 1.02 | 0.64 to 1.7 | up | [79], [82], [84] |
| LONRF3 | 3 | 4 | 5.78e-05 | 0.96 | 0.62 to 1.52 | up | [79], [84], [82] |
| CXCL3 | 3 | 3 | 8.65e-05 | 1.58 | 0.64 to 2.52 | up | [79], [83], [84] |
| CTSK | 3 | 3 | 4.54e-04 | 1.15 | 0.73 to 1.55 | up | [79], [82], [84] |
| ITGA4 | 3 | 3 | 1.30e-03 | -1.07 | -1.76 to -0.65 | down | [79], [82], [84] |
| SATB1 | 3 | 3 | 1.70e-03 | -1.01 | -1.51 to -0.75 | down | [81], [82], [84] |
| GLIPR1 | 3 | 3 | 1.90e-03 | -1.00 | -1.29 to -0.63 | down | [82], [83], [84] |
| MAFF | 3 | 3 | 1.93e-03 | 0.75 | 0.72 to 0.82 | up | [79], [82], [84] |
| CD83 | 3 | 3 | 2.20e-03 | 0.73 | 0.6 to 0.9 | up | [79], [82], [84] |
| PIM1 | 3 | 3 | 2.46e-03 | 1.41 | 0.67 to 2.88 | up | [79], [82], [84] |
| RDX | 3 | 3 | 2.66e-03 | 0.87 | 0.62 to 1.34 | up | [79], [82], [84] |
| SDC4 | 3 | 3 | 2.81e-03 | 0.72 | 0.64 to 0.84 | up | [79], [82], [84] |

Note:

Excerpt from the ranked list of common DEG across at least three transcriptome studies (PECO 1a) arranged according to the number of studies and experiments in which they were expressed in the same direction, position achieved by the RRA method (= Score) and mean fold change (= FC.Mean) [17]

^a Various tests within a study

2. Results

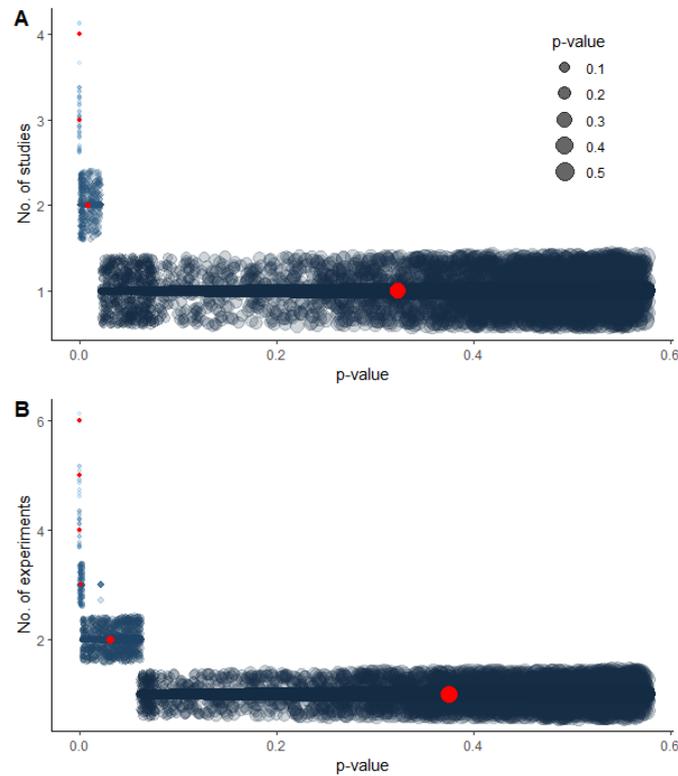


Figure 2.7: Scatterplot showing how the p-values of each gene from DEG lists of multiple studies (PECO 1a) calculated via RRA method (lower values are given at the top of the list) are distributed compared to the number of studies (A) and experiments (B) in which a gene is the same. Genes that were multiple reported in studies/experiments had lower p-values. The red dots marks the mean p-value. Jittering, i.e., adding a random variation to the position of each dot, was applied to handle overlapping.

The number of DEG reported varied considerably between the experiments of different studies, ranging from 13 to 5098. When trends in gene expression were investigated according to cell type, dose and time within one study under the same conditions, the authors obtained different results. For example, the study by Tilton et al. showed a concentration and time-dependent response of THP-1 cells, whereas another cell line (small airways epithelial cells) expressed most genes at the shortest time point, suggesting a cell line-specific response [84]. Furthermore, in another study a time dependence in gene expression was observed [83].

Gene Ontology and Pathway Enrichment Analyses In three separate studies, the impact of transcriptome alterations on biological processes was evaluated through gene ontology and/or pathway enrichment analyses [17]. As a result,

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101 distinct GO terms and 112 unique pathways were reported [17,79,83,84]. Categorization of GO terms/pathways to broader terms revealed that both general processes such as signal transduction and metabolism but also more specific ones like the cellular response to stimulus/stress were altered (see Figure 2.8). Genes involved in inflammatory/immune responses or programmed cell death and cell cycle regulation were also commonly affected in cells exposed to titanium particles. In addition, pathways of the innate rather than adaptive immune system were enriched such as ‘Toll-like receptor signaling’, ‘Phagocytosis’ or ‘Cytokine signaling’. Phagocytosis was also recognized via image analysis or mass spectrometry in studies with macrophages and osteoblasts [79,81,90]. Various transcriptome studies showed that internalized titanium particles modulate the transcellular transport or cell adhesion of endothelial cells or deform the cytoskeleton of osteoblasts and cause an immune/inflammatory response by upregulation of cytokine gene expression in nearly all analyzed cell lines [17,79,81–84].

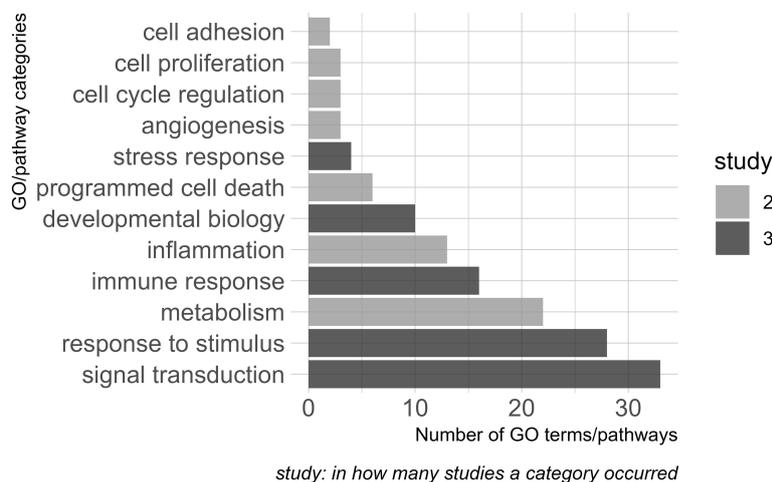


Figure 2.8: Bar plot representing the distribution of altered biological processes reported in transcriptome studies examining titanium exposure of cells (PECO 1a), adapted from Freitag et al., 2023 with permission of the publisher [17]

Self-conducted DAVID functional enrichment analyses of genes expressed equally in a minimum of two studies revealed a similar pattern of modified cellular processes

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as in the studies stated above, whereby inflammatory and immune responses predominated [17]; pathways such as ‘TNF- α signaling’, ‘NOD-/Toll-like receptor signaling’ or ‘MAPK signaling’ were impacted [17]. The Figures 2.9 and 2.10 contain “the top ten GO terms and pathways with lowest FDR” [17].

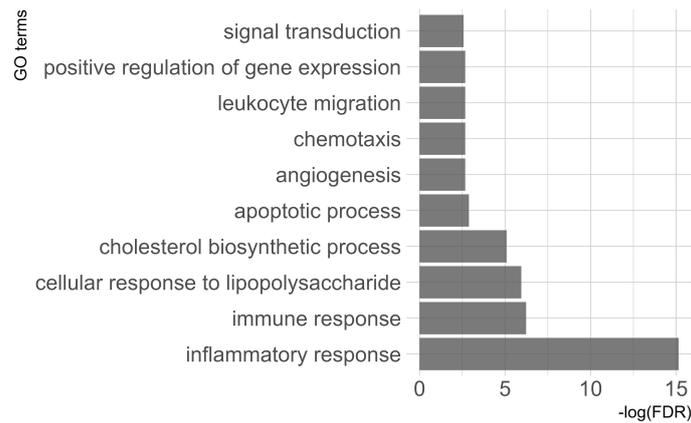


Figure 2.9: The top 10 enriched GO categories of common expressed genes in at least two transcriptome studies examining titanium exposure of cells (PECO 1a) via DAVID functional annotation chart tool, adapted from Freitag et al., 2023 with permission of the publisher [17]

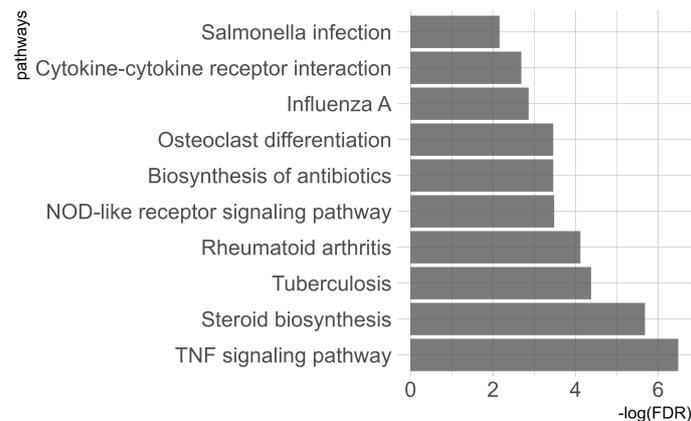


Figure 2.10: The top 10 enriched pathways of common expressed genes in at least two transcriptome studies examining titanium exposure of cells (PECO 1a) via DAVID functional annotation chart tool, adapted from Freitag et al., 2023 with permission of the publisher [17]

2. Results

Proteome Studies Each of the three studies stated a statistically significant alteration in the proteome when exposed to titanium regardless of variations in cell lines and experimental settings (see Table 2.1) [17,78,83,84]. The mean value of DEP across the ten experiments within the three papers was about 61 proteins. In one study, different sizes of titanium particles were used in various experiments, whereas particles with lowest dry size (highest hydrodynamic sizes) showed highest proteomic changes [83].

Overlap of Differentially Regulated Proteins In one study [84], the names of the differently regulated proteins were not reported, thus the DEP lists from only two studies could be compared [17,78,83]. Thereby, nine proteins (2,8%) were present in both studies and 5% of the 320 distinct proteins overlapped in two experiments (see Table S2 in Supplemental Material) [17]. Among these, 11 proteins displayed consistent expression patterns in two experiments, primarily showing a decrease in their expression levels (see Table 6) [17]. Following three proteins were equally downregulated in both studies: CCT8, HSP90AB1, TUBA1B [17]. Additionally, cytoskeletal proteins like tubulin and actin as well as proteins belonging to families like annexin or peroxiredoxin, along with components of the 26s proteasome, showed downregulation associated with titanium in the two studies [17].

Since there was no information provided regarding the number and names of all identified proteins in both proteomic analyses, Monte Carlo simulations and RRA could not be applied to test the significance of these results [17].

2. Results

Table 2.5: Ranked list of common DEP across proteome studies of PECO 1a, adapted from Freitag et al., 2023 with permission of the publisher [17]

| Symbol | Study | Experiment ^a | FC.Mean | FC.Range | Direction | Reference |
|----------|-------|-------------------------|---------|----------------|-----------|------------|
| CCT8 | 2 | 2 | -2.47 | -3.96 to -0.98 | down | [78], [83] |
| HSP90AB1 | 2 | 2 | -2.21 | -3.49 to -0.94 | down | [78], [83] |
| TUBA1B | 2 | 2 | -1.90 | -2.87 to -0.93 | down | [78], [83] |
| XRN2 | 1 | 2 | -1.92 | -2.64 to -1.2 | down | [83] |
| RPS26 | 1 | 2 | -1.79 | -2.41 to -1.17 | down | [83] |
| GC | 1 | 2 | 1.69 | 1.41 to 1.98 | up | [83] |
| AFP | 1 | 2 | 1.53 | 1.38 to 1.68 | up | [83] |
| TSN | 1 | 2 | -1.06 | -1.34 to -0.78 | down | [83] |
| GSTP1 | 1 | 2 | -1.03 | -1.42 to -0.64 | down | [83] |
| GNB2 | 1 | 2 | -0.91 | -1.12 to -0.7 | down | [83] |
| PTMS | 1 | 2 | -0.86 | -0.99 to -0.72 | down | [83] |

Note:

Ranked list of common DEP across two proteome studies (PECO 1a)

arranged according to the number of studies and experiments in which they were expressed in the same direction and mean fold change (= FC.Mean)

^a Various tests within a study

Gene Ontology and Pathway Enrichment Analyses Enrichment analyses for pathways and gene ontology were performed in each of the three proteome studies, but with different methods [17]. The results were similar to those of the transcriptome studies (see Figure 2.11) [17]. For example, modified processes related to the immune system or metabolism are in the top categories in both proteome and transcriptome studies. Figure 2.12 presents the subcategories of metabolic processes affected by titanium of all proteome/transcriptome studies with carbohydrate, lipid, protein, and amino acid metabolism being prevalent. One study using both transcriptome and proteome analyses also revealed similarities in the altered biological processes by titanium in the two omics datasets; for instance, ‘apoptotic nucleus process’ (cell cycle arrest/genomic instability) and ‘inflammation’ were common processes [17,84]. In addition, proteomic studies commonly reported on specific biological processes such as the response of cells to oxidative stress after exposure to titanium [17]. Although no increased levels of reactive oxidative species (ROS) occurred in other experiments, proteomic analyses showed proteins

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mainly from the peroxiredoxin, SOD, or annexin family to be affected after titanium exposure [17,78]. Another study found an association between titanium-induced oxidative stress response and inflammatory cytokines such as TNF- α [17,84]

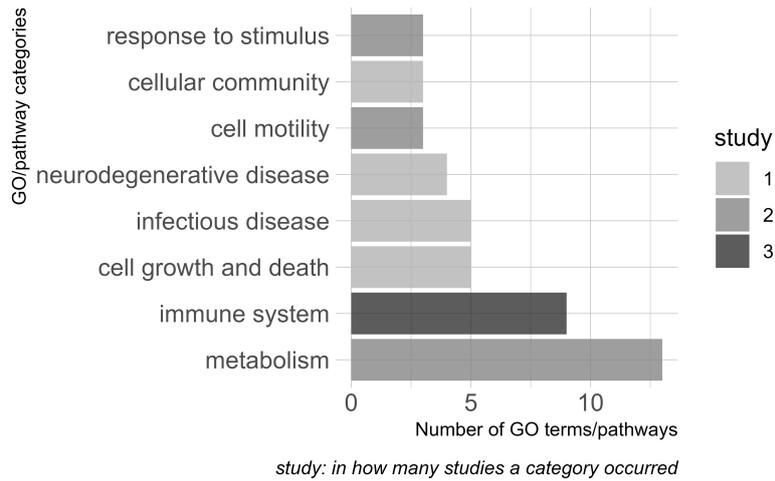


Figure 2.11: Bar plot representing the distribution of altered biological processes reported in proteome studies examining titanium exposure of cells (PECO 1a), adapted from Freitag et al., 2023 with permission of the publisher [17]

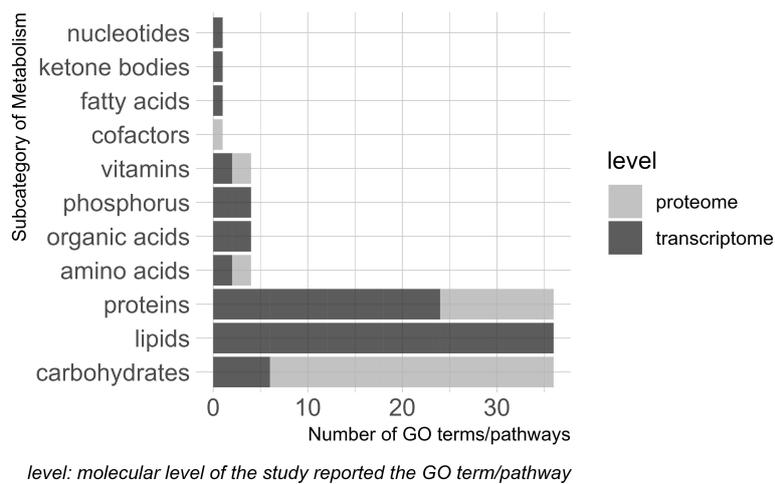


Figure 2.12: Bar plot representing the distribution of subcategories of metabolic processes reported in proteome and transcriptome studies examining titanium exposure of cells (PECO 1a)

2. Results

Epigenome Studies Only three studies were found that investigate the impact of titanium particles on epigenetics in oral-related cells (i.e., macrophages) [17]. Among these, one study focused on miRNAs [87], the others analyzed global DNA methylation changes [88,89]. 102 miRNAs were differentially expressed in response to TiO₂ nanoparticles in THP-1 cells after 6 and 24 hours with the majority being members of the let-7-miRNA family [87]. However, a separate analysis of their target genes and biological function was not performed.

No change in global DNA methylation (5mC) and hydroxymethylation (5hmC), respectively, was detected in macrophages exposed to TiO₂ nanoparticles [88,89]. The methylation status of transposable elements, LINE-1 and Alu, also did not change with titanium exposure. Additional PCR experiments measuring the gene expression levels of DNA methylation machinery demonstrated a significant decrease in DNA methyltransferases (DNMT1, DNMT3A, DNMT3B, and UHRF1) following exposure to titanium [17,88]. Another study identified dose-dependent global DNA hypomethylation in peripheral blood monocytes due to TiO₂-NP exposure at non-toxic concentrations [17,89].

2.1.5.2 PECO 1b

No study using omics techniques examined the cellular exposure to titanium ions [17].

2.1.5.3 PECO 1c

“In human cell cultures [P], how do molecular signatures [O] change upon exposure to titanium particles/solution [E] compared to other metallic particles/solutions [C]?” [17]

Four studies were identified that exposed cells to titanium and various other metals, including two transcriptome studies [85,86] and two epigenome studies [87,88]. However, none of these studies directly compared TiO₂ with other metals [17].

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Transcriptome Studies Each study examined the transcriptomic changes of different cells caused by TiO₂ and ZnO [85,86], and one study additionally investigated the effect of silver on the transcriptome [85]. They showed that different metals (ZnO and Ag) had particle-specific as well as common effects in gene expression [85,86]. In both studies, the effect of zinc/silver particles on the transcriptome was greater than that of titanium [85,86,91], with no significant alteration in gene expression by TiO₂ [85,86]. In one study metal ions (Ag) were tested in addition to particles showing weaker responses in the transcriptome [85]. Common processes in cells exposed to other metals than TiO₂ were found across the trials. For instance, inflammation and immune response with changes in gene expression of chemokines or cytokines as well as genes associated with pattern recognition like toll-like receptors [85,86]. Of particular note is the upregulation of genes related to the family of metallothioneins and the GO term ‘Unfolded protein response’, which was associated to (oxidative) stress response, as it has been observed in various experiments (different cell/particle types) within each study [85,86].

Epigenome Studies Two studies investigated the epigenetic changes caused by metal particles such as titanium oxide, copper oxide, and silver in THP-1 cells over a maximum of 24 hours [87,88]. Ndika et al. showed that the majority of DE miRNAs (102) were induced by TiO₂-NP, followed by Ag and ZnO-NP. However, silver nanoparticles caused the strongest change in miRNA expression (highest fold changes) [17,87]. 9.6% of all DE miRNAs overlapped between TiO₂ and Ag nanoparticles, while 12% were identical for TiO₂ and ZnO nanoparticles [17]. Correlation analysis of miRNA and mRNA expression across all metal types demonstrated that the genes with the strongest correlations were associated to processes such as “cell cycle regulation, inflammatory response, and response to metal ions” [17]. However, titanium had only a weak correlation to the potentially co-regulated seven miRNAs and 182 genes that were most affected by Ag and ZnO particles [87]. Neither TiO₂ nor CuO nanoparticles induced global epigenetic changes in sense of global DNA hypomethylation. In other experiments, hypermethylation

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of repetitive elements like LINE-1 or Alu by CuO particles and alterations in the expression of genes of the DNA methylation machinery through metals were observed [17,88].

2.1.5.4 PICO 1d

The combined effect of titanium and oral bacteria or their pathogen-associated molecular patterns (e.g., LPS) has not been investigated in any omics study [17].

2.1.5.5 PICO 2a

“In animals [P], how do molecular signatures [O] differ between healthy dental implants [E] compared to healthy teeth [C]?” [51]

Two separate studies on rats revealed unique gene expression patterns in the soft tissues surrounding dental implants and in periodontal tissue [51,92,93]. In these studies, the number of DEG with more than twofold change reached 1279 and 2219 in the peri-implant tissue four weeks after implantation, respectively [51]. There was no overlap between the two studies in any of the 39 reported DEG that showed more than a fivefold change in expression [51]. Due to the absence of functional enrichment analyses in either study, a thorough comparison of the changed biological processes in periodontal and peri-implant tissue was not feasible [51]. Nevertheless, both studies showed that oxidative stress linked to reactive oxygen species is present following implantation [51]. At the transcriptome level, one article revealed a downregulation of superoxide dismutase 3 (SOD3), leading to an excess of ROS, confirmed via immunohistochemistry [51,92]. The second study reported an upregulation of the gene encoding lactoperoxidase (LPO) in the presence of the implant, presumably to reduce the damage caused by ROS expression [51,93].

2.1.5.6 PICO 2b

“In animals [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [E] compared to dental implants without an inflammation [C]?” [51]

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In two split-mouth studies involving Labrador dogs, 38 and 65 DE miRNAs were identified in the soft tissue of ligature-induced peri-implantitis relative to healthy peri-implant sites [51,94,95]. The RT-PCR validation of selected miRNA expression profiles revealed a significant decrease of let-7g and miR-27a, along with an increase in miR-145, observed consistently in both studies [51,94,95]. Only one of the studies conducted GO enrichment analysis, demonstrating an association between DE miRNAs and processes linked to inflammatory response and bone metabolism and thus could be related to the pathology of peri-implantitis [51,94]. In particular, an enrichment was detected among the upregulated target genes associated with the MAPK, NF- κ B, TGF- β , and toll-like receptor signaling pathways[51,94].

2.1.5.7 PICO 2c

No omics studies were available in animals comparing induced peri-implantitis and periodontitis [51].

2.1.5.8 PICO 3a

Human omics studies comparing healthy dental implants to healthy teeth were not present [51].

2.1.5.9 PICO 3b

“In systematically healthy, non-smoking humans [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [E] compared to dental implants without an inflammation [C] a minimum of one year after implant placement [T]?” [51]

In a discovery cohort study, the transcriptomes of inflamed and healthy implants were compared using three and four tissue samples respectively [51,96]. In diseased tissues, there was a significant increase of at least twofold in four genes encoding proteins: BLOC1S4, CDK12, WASH1, and DNAJC28; these genes are involved in functions such as intracellular vesicle movement and trafficking, transcriptional

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regulation of DNA repair, and stabilization genes or oxidative stress [51,96]. Remarkably, signaling pathways related to keratinization, oxidative stress and local immunity exhibited high expression levels in healthy as well as inflamed peri-implant samples, indicating a high baseline level of oxidative stress and inflammation in tissue surrounding implants [51,96]. However, “bacterial system response genes were not upregulated in peri-implantitis versus healthy sites” [51,96].

2.1.5.10 PICO 3c

“In systemically healthy, non-smoking humans [P], how do molecular signatures [O] differ in inflammatory lesions around dental implants [E] and around periodontally compromised teeth [C] a minimum of one year after implant placement [T]?” [51]

Four studies were identified that analyzed the transcriptomes of gingival tissue affected by peri-implantitis and periodontitis along with healthy periodontal tissue (in two articles) [51,75,97–99]. Three of these studies additionally performed epigenome analyses, with two studies focusing on the expression pattern of long non-coding RNAs (lncRNAs) and miRNAs [75,99] and one on the measurement of DNA methylation [97].

In each study, unique gene expression patterns for the two diseases were observed at the mRNA as well as the lncRNA/miRNA levels [51]. Moreover, peri-implantitis specimens showed a greater discrepancy with the healthy state than periodontitis ones [75,99]. Differences in the immune response between peri-implantitis and periodontitis at the RNA level were found in almost all studies [75,98,99]. For example, the lncRNA (DPP-10 AS-1) with lowest expression in peri-implantitis versus periodontitis samples was suggested by Liu et al. to be linked to TH2 type immune response by IgE, which was also associated with titanium particles [51,75]. The same study revealed that B and plasma cell immune responses predominate in peri-implantitis [51,75]. Furthermore, the GO enrichment analysis in the study by Zhou et al. highlighted that the gene expression linked to innate immune response, response to metal ion or reactive oxygen species and defense response were higher in

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peri-implantitis than in periodontitis as well as healthy periodontal tissues [51,99]. In the same study, the competing endogenous (ceRNA) network with 16 microRNAs, 91 lncRNAs and 377 mRNAs nodes identified six genes – FAM126B, SORL1, PRLR, CPEB2, RAP2C and YOD1 – as the core of the network [99]. These genes primarily showed correlation pathways related to proliferation, processes associated with ROS, infection, and host stress process [51,99]. Most of the target genes of the five core lncRNA nodes were tumor-related receptor proteins with important roles in the pathology of many diseases [99]. Another trial examining alterations in lncRNA expression hypothesized that titanium-induced microtubular dysfunction was regulated by lncRNAs [51,75]. Moreover, there was a correlation between 100 lncRNAs and RANKL as well as heightened RANKL/OPG ratio which is linked to an accelerated bone resorption rate, as also evident in PCR experiments [51,75]. Additionally, the increased gene expression levels of BMP-5 and the upregulation of the osteoclast differentiation pathway in peri-implantitis than periodontitis tissues pointed to faster bone resorption in peri-implantitis [51,75].

The number of DEG (up to 2892) across the studies was not comparable due to selective reporting (only 86 DEG published in total) and no overlapping gene between the studies was found (see Table S3 in Supplemental Material) [51]. Nevertheless, in all studies, genes belonging to the matrix metalloproteinase family (MMP7, MMP12, MMP14) were amongst the most frequently reported DEG, with one upregulated (MMP14) and two downregulated ones (MMP7, MMP12) [51,75,97–99]. In response to heightened MMP14 levels, Roediger et al. observed an increase in the “expression of tissue inhibitors of the matrix metalloproteinases (TIMP1 and TIMP3) [along with] other proteases with a role in degradation of extracellular matrix in peri-implant tissue (DAM15, ADAM17, ADAMTS6 and Cathepsin D and S)” [51,98].

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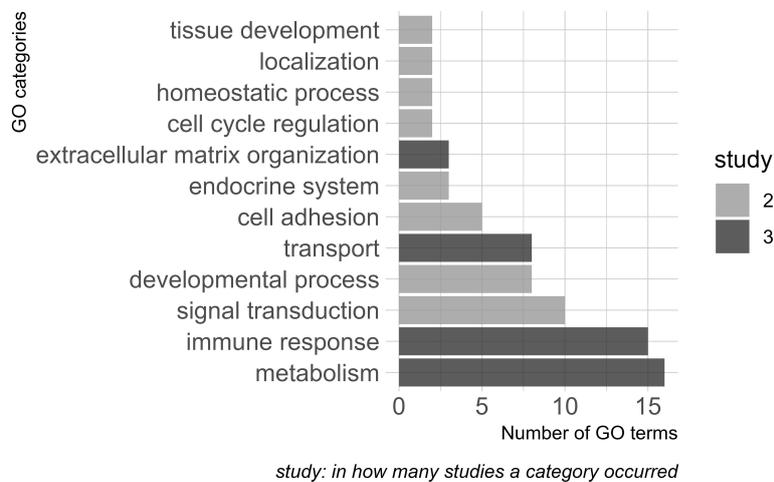


Figure 2.13: Bar plot representing the distribution of altered biological processes reported in transcriptome studies comparing peri-implantitis with periodontitis (PICO 3c), adapted from Spinell et al., 2023 with permission of the publisher [51]

Gene ontology and/or pathway enrichment analyses were carried out in all studies, but the published results of one study were not suitable for comparative analysis since the enriched GO terms were reported for periodontitis compared to peri-implantitis [97]. The categorization of GO terms/pathways indicated that mainly processes in metabolism and immune response differ in peri-implantitis compared to periodontitis, but also changes in processes in extracellular matrix organization and cell adhesion were commonly mentioned (see Figure 2.13) [51]. Again, the MAPK and Toll/NOD-like receptor signaling pathways were frequently enriched in peri-implantitis tissues relative to periodontitis tissues [51].

Epigenome Studies Besides the miRNA and lncRNA changes mentioned above, solely one study additionally examined methylome alterations between periodontitis and peri-implantitis tissues [51,97]. Cho et al. detected 77 differentially methylated genes (43 hyper-, 34 hypomethylated)[97]. However, there were no GO terms found to be statistically significant for these genes [51,97].

2.2 Comparison of a Large-Scale Transcriptome Study and Results of the Systematic Review

2.2.1 Comparison with Transcriptome Studies Examining the Differences between Peri-Implantitis and Periodontitis

Clustering of terms with similar biological meaning based on GO terms and pathways (via DAVID functional annotation clustering tool) of DEG between peri-implantitis and periodontitis revealed common altered biological processes between a study with a large sample size and those with considerably smaller ones [75,98,99], specifically: immune system (cytokine response), metabolic processes and cell adhesion (see Figure 2.14). By contrast, more specific GO terms (292) and pathways (35) of enrichment analysis (via DAVID functional annotation chart tool) showed little overlap as only three GO terms and seven pathways were equal to the selective reported GO terms (55)/pathways (48) of included studies in the systematic review [75,98,99] (see Table 2.6). This is also reflected at the DEG level, where only 6.8% of the DEG of the large-scale transcriptome study overlapped – with same direction of expression – with the DEG list generated from the raw data of a study included in the systematic review (see Table S4 in Supplemental Material) [75]. An overlap in the functional enrichment analysis performed on all DEG of these two studies was also not apparent in the comparative analysis via the `clusterProfiler` R package.

2. Results

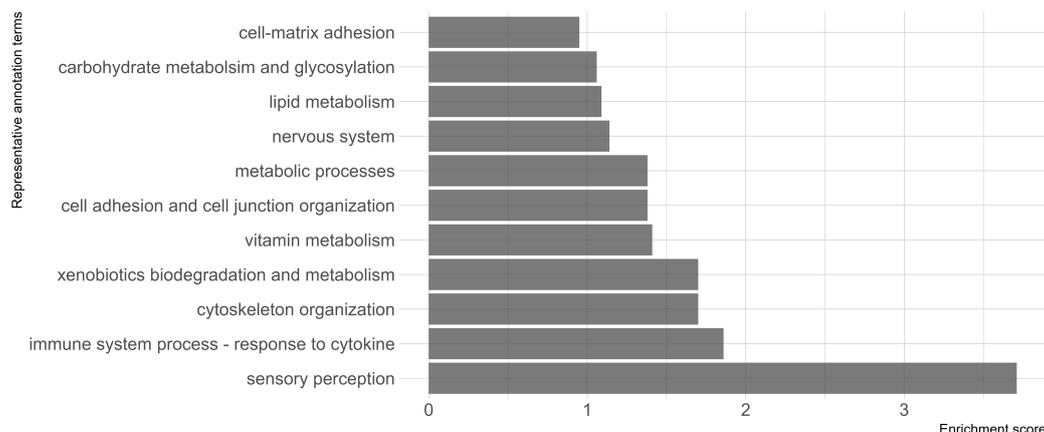


Figure 2.14: The most enriched functional annotation clusters (via DAVID functional annotation clustering tool) of DEG ($FC \geq 1.5$ and adj. p -value < 0.05) of a large-scale transcriptome study comparing peri-implantitis with periodontitis

Table 2.6: Common GO terms and pathways found in enrichment analyses between the large-scale transcriptome study and transcriptome studies included in the systematic review (peri-implantitis vs. periodontitis)

| ID | GO term/pathway |
|------------|--|
| GO:0030198 | extracellular matrix organization |
| GO:0045444 | fat cell differentiation |
| GO:0030049 | muscle filament sliding |
| hsa04060 | cytokine-cytokine receptor interaction |
| hsa05146 | amoebiasis |
| hsa00980 | metabolism of xenobiotics by cytochrome p450 |
| hsa00982 | drug metabolism - cytochrome p450 |
| hsa04512 | ecm-receptor interaction |
| hsa00350 | tyrosine metabolism |
| hsa04310 | wnt signaling pathway |

2.2.2 Comparison with Transcriptome Studies Examining the Impact of Titanium Particles on Cells

A small overlap in the functional profile of DEG expressed in peri-implantitis versus periodontitis and in cells exposed to titanium was present in the over-representing analysis using the `clusterProfiler` algorithm (see Figure 2.15). In particular, GO terms related to cytokine/chemokine response and signal transduction as well

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as pathways linked to inflammatory host response mediated by cytokines were commonly enriched in peri-implantitis affected tissues and titanium exposed cells. Of the 2846 DEG ($FC \geq 1.5$ and adj. p-value < 0.05) between peri-implantitis and periodontitis, 188 (6.6%) genes were also up-/downregulated in the same direction in cells treated with titanium particles compared to cells without titanium exposure (see Table S5 in Supplemental Material). For instance, chemokines such as CXCL2, CCL3, CCL7 and family members of metallothionein (MT2A, MT1G, MT1H) were concordantly upregulated.

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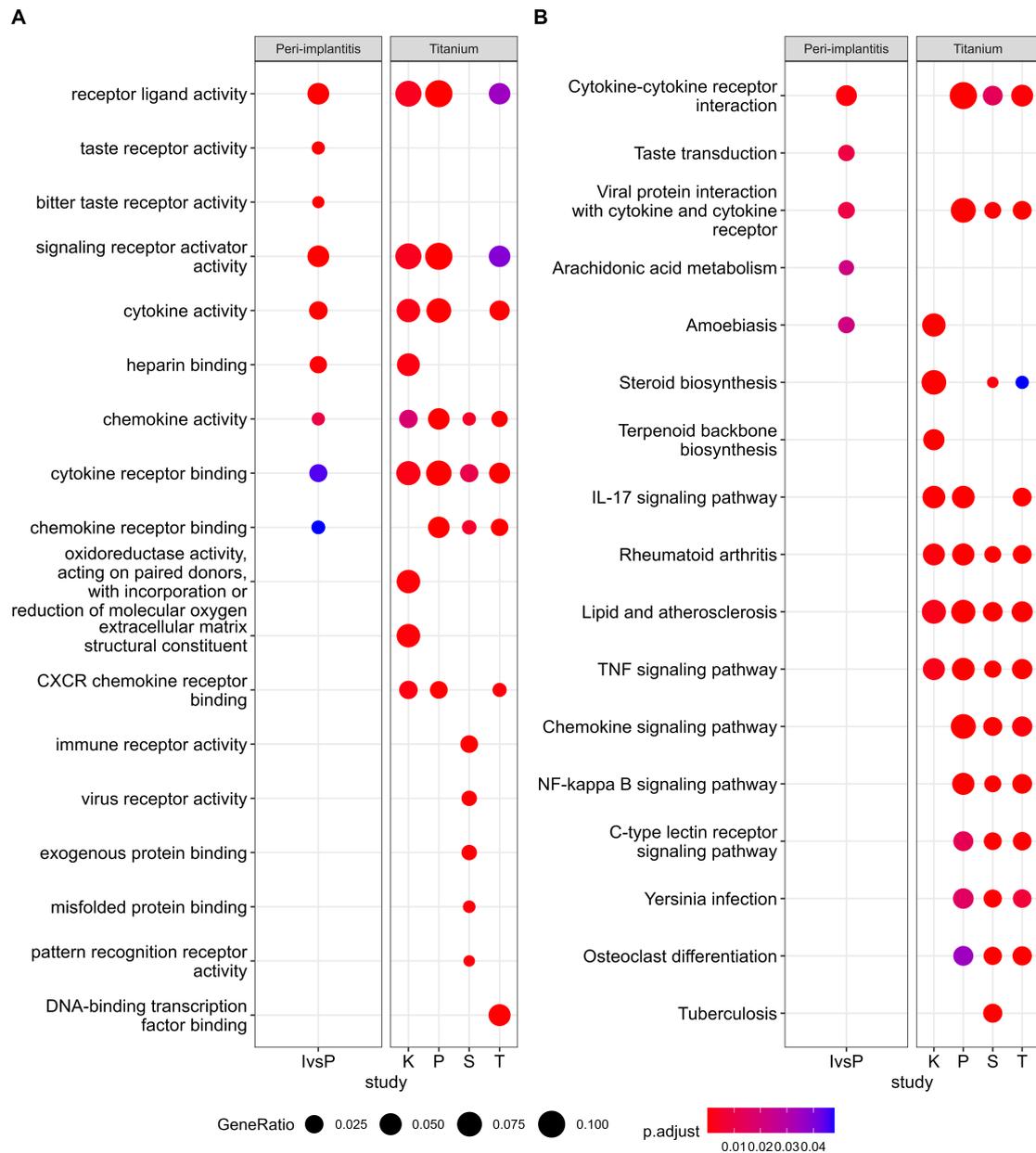


Figure 2.15: Dot plot comparing the functional enrichment results (A: GO terms, B: pathways) of DEG expressed in peri-implantitis than periodontitis and in cells exposed to titanium displayed in two facet panels. The x-axis represents the respective studies: large-scale study examining the transcriptomic difference between peri-implantitis and periodontitis (IvsP) and the transcriptome in vitro studies with titanium exposure included in the systematic review: Kuruvilla et al. (K), Pajarinen et al. (P), Silva-Bermudez et al. (S), Tilton et al. (T). The adjusted p-values of the functional enrichment analysis indicate low (red) or high (blue) enrichment for each category. The size of the dots reflects the number of genes matching a GO term/pathway relative to all inputted genes with a GO/pathway annotation.

3

Discussion

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3.1 Systematic Review

The reported histopathological and clinical differences between peri-implantitis and periodontitis were verified in omics studies, primarily at the transcriptome level, but also at the epigenome level [51]. Apart from differences in attachment structures

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of implants and teeth [14,24], the implant material itself might play a role in the development and progression of peri-implantitis [51].

Multiple studies indicate that titanium particles induce changes in the proteome and transcriptome of oral cells, although there are studies that could not measure altered gene expression. Potential reasons for these discordant results of transcriptome analyses might be attributed to the following limitations of gene expression data. In addition, the cellular effect of titanium particles and ions on biological processes that were frequently affected in the transcriptome and proteome analyses will be discussed. The possible different and common effects of various metals will also be highlighted as well as a potential synergistic impact of titanium and bacteria. Based on common processes that are altered by titanium and in peri-implant compared to periodontal tissues, the effect of titanium wear debris on peri-implantitis will be discussed subsequently.

3.1.1 Limitations of Gene Expression Data

3.1.1.1 Biological and Experimental Variation

In addition to differences in gene expression between cell lines and the tissue from which they originate [100], the transcriptome also differs between single cells of similar origin. For example, most gene expression changes induced by zinc oxide in immune cells (Jurkat T cells, HMDM, MDDC) were found to be cell type-specific [86]. Since two of these cell lines were not used in other studies included in the systematic review, the cell line-specific response may explain the unchanged gene expression after titanium exposure in this study [86]. Even the same cell line but with different polarization (M0, M1, M2 macrophages) showed distinct transcriptome (and proteome) profiles after exposure to titanium dioxide [79,82].

Besides these biological factors, differences in study design can also lead to different results [101]; with respect to *in vitro* studies, this concerns the different combinations of particle type (size, crystal phase), concentration, and exposure time. For instance, six titanium particles with the same elemental composition but varying physical-chemical properties showed quite different responses in a study

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investigating changes in the transcriptome of colon cells, i.e., an increase in the number of DEG due to increased agglomerated particle sizes [102].

3.1.1.2 Technical Variation

Furthermore, there are technical, non-biological causes of variation in omics data between different studies, also referred to as ‘batch-effects’. In the case of microarray technologies, these include the variability in the isolation of mRNA or hybridization onto the microarray as well as ambient conditions during analysis in different laboratories [103].

Differences in the microarray platforms used can also influence the results across studies [101]. Mainly, microarrays can be classified either by the method of manufacture, by the number of samples that can be analyzed simultaneously, or by the length of the base pairs immobilized on the microarrays to target the mRNA from the samples [104]. The latter are distinguished into two types: ‘oligonucleotide arrays’, which consist of short probes with 50 bps or less and ‘complementary DNA (cDNA) arrays’, which use relatively longer DNA molecules [104]. Both were applied in the selected studies for this systematic review as well as microarrays constructed with different methods such as synthesized arrays (e.g., Affymetrix) or self assembled arrays based on beads (e.g., Illumina) [105]. Because various platforms differ in the number and types of genes examined, studies can reach different results. For example, Tuomela et al. conducted a second analysis with another microarray platform on cells exposed to ZnO nanoparticles [86]. They showed an average consistency of 76% with a wide range of 30.6-100% of genes with same direction of expression and significant adjusted p-value between different experiments [86]. Nevertheless, the MicroArray Quality Control (MAQC) project revealed a generally high degree of concordance in inter-laboratory and cross-platform comparisons, which depended mainly on the statistical criteria applied for determining differentially expressed genes rather than the chosen platforms [106].

A few studies included in this systematic review used RNA sequencing technologies [83,87,94,95,97,99]. This method offers the advantage of examining the

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entire transcriptome, thus identifying a larger number of DEG, and reducing noise by direct measurement of gene expression [107]. In contrast, microarrays indirectly profile only predefined genes [107]. Nevertheless, a high level of concordance in DEG (>75%) and pathway analysis between these methodologies was reached in a comparative study by Rao et al. [107].

3.1.1.3 Data Transformation and Analysis

Pre-processing methods like background correction and normalization of raw fluorescence data of images obtained from microarrays as well as different statistical analysis methods with varying cutoff criteria leads to variance in measured gene expression [108]. For instance, three different normalization methods of microarray data with same experimental conditions produced about 30% concordant DEG in the results of at least two normalization methods [109]. Different statistical tests can be applied to detect DEG between two groups by considering their variance resulting in different gene lists [110]. A widely used, simple and easily interpretable method is the gene-specific *t*-test [110]. However, it is increasingly being replaced by more advanced statistics such as `limma` (linear models for microarray and RNA-Seq data) [111] because of problems with skewed variance estimation and low power for small sample sizes [110]. A comparative study of eight statistical tests such as parametric (e.g., ANOVA, Welch's *t*-test, `limma`) and non-parametric (e.g., Wilcoxon's test, SAM test) approaches indicated that tests with similar variance modeling strategy (same variance between groups of samples or gene-by-gene variance estimation) reached similar results [110]; however, results by significance analysis of microarrays (SAM) showed no reproducible behavior and SAM, unlike other methods, was unable to find any DEG in a re-sampling approach [110]. This could be a possible reason why, in contrast to other studies, an *in vitro* study using the SAM analysis did not detect any significant DEG after titanium exposure [80]. As shown in section 2.1.4, some studies provided insufficient information on the statistical tests used, which complicated their comparison. In addition, the cutoff criteria of determining significantly differentially expressed genes – fold change values (showing

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how much the expression values differ between test and control group) and p-values (reflecting the significance level according to the statistical test applied) – have a considerable impact on the length of DEG lists [108]. Hence, the stringency of the adjustment approach compared to other studies, with a Benjamini-Hochberg adjusted p-value of less than 0.01 in the study by Poon et al., may be one reason why no significantly DEG were elicited by titanium exposure in this study [85]. Because a large proportion of overlapping genes between lists of different studies may be just below the significance level, this can lead to apparent discordance between lists [101]. Nevertheless, the MAQC project indicated that DEG lists limited by both fold change and p-values are more reproducible than lists based on p-value solely, which was validated in a rat toxicogenomics data set [106]. The combination of these cutoff criteria can also reduce the influence of different normalization techniques on the reproducibility of DEG lists [106]. Thus, both cutoff criteria were used in the compilation of the DEG lists for this systematic review.

3.1.1.4 Sample Size

In addition to the aforementioned aspects, the small sample sizes, especially of the selected *in vitro* studies, should be taken into account. No study performed “a priori power analyses to justify the sample size” [17]. Since the rate of false positive results and missed true signals (false negative results) increases rapidly as the sample size decreases, the chosen sample size has a considerable effect on the number and significance of the DEG [17,48,112]. For instance, the low overlap in gene signatures of 8.0% in at least two studies with titanium-exposed cells and no overlap of most deregulated genes in *in vivo* studies raise concerns about its reliability and robustness. In particular, the selected *in vitro* study by Pajarinen et al. only used one sample per test and control group, which drastically increases the risk of bias of this study [79]. To obtain reliable and reproducible results, future research should consider an estimate of the sample size. Corresponding methods for microarray or RNA sequence experiments can be found in the literature, but are rarely applied so far [113,114].

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3.1.1.5 Random Noise

To generate gene expression values in terms of relative intensities, thousands of genes are measured simultaneously for each sample [115], resulting in a large number of variables (i.e., gene expression values for each gene) but for a small number of samples (i.e., biological replicates). This is known as the ‘large p, small n’ problem [116]. Performing statistical tests for each gene would dramatically increase the false positive results [101]. For example, 20,000 tested null-hypothesis with a 0.05 probability of rejecting a true null hypothesis (= type I error) would generate an average of 1,000 false positives [101]. Therefore, adjustments for multiple testing such as the controlling the false discovery rate (FDR) via Benjamini-Hochberg procedure are required [116]. This method was used in most of the selected studies, whereas statistical tests were not well described, especially in the animal and also in the human studies [79,81,92–98]. Thus, combining the results of multiple studies should provide the advantage that genes with a ‘real’ change in expression, i.e., due to titanium, are more likely to be detected than the false positive genes [101].

3.1.2 Integration of Gene Expression Data

To integrate gene expression data of several studies there are two general strategies: analyzing raw data of high-throughput experiments or working with the reported results in each study such as DEG lists [63].

Microarray meta-analysis working with raw data can be classified according to Hamid et al. in ‘early’, ‘intermediate’ or ‘late’ stage of integration of the raw files [117]. When raw data are normalized together, e.g., through cross-platform normalization, and statistical analyses are applied to the merged dataset, this is considered integrated at the late stage [118]. In contrast, final statistic results are combined from different studies at early stage [118]. However, the application of this methodology is limited to the availability of raw data, which is often not provided, and does therefore reduce the number of studies that can be compared. Even if DNA microarray data are deposited in online databases such as GEO or ArrayExpress, in about a quarter of the cases they are not suitable for meta-analysis

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due to low quality [119]. For example, Roediger et al. deposited only two of the 16 samples used at the GEO database in a format that could not be easily analyzed by GEO2R [98]. Moreover, Liu et al. provided non-annotated data without information about the annotation they used [75]. This does not correspond to the guideline for an adequate description of how microarray data are generated (MIAME: Minimum Information About a Microarray Experiment) [120].

Although meta-analysis of raw microarray data provides advantages (in terms of statistical power) [121], the integration of reported results increases the number of suitable studies, avoids normalization problems that are caused by different microarray platforms [63], and is less elaborate in most cases.

Because few studies provided raw data stored in online databases, published DEG lists and self-generated DEG lists based on available raw data were used to obtain most of the information. Two distinct methods (see section 1.1.6) were adopted to receive a list of genes that were commonly reported between the studies and are most significant in association with titanium exposure. Selecting genes that were expressed in the same direction in multiple studies could produce false positives as the overlap could occur by chance. Therefore, a simulation of randomly selected common genes with 10,000 permutations were performed to compare the amount of empirical overlap with the overlap expected by chance [59]. Nevertheless, this approach is quite simplistic with regard to the complexity and variability of gene expression [122]. Lawhorn and co-workers demonstrated via computer simulations that this randomly expected overlap is usually underestimated [122]. This leads to overestimates of significant overlap between DEG lists if all genes are assumed to be identical in their degree of expression control [122]. However, as robust rank aggregation reached similar results especially for the multi-study genes, mainly these genes could be influenced by titanium.

Based on how microarray data is created (see section 3.1.1), comparing already constructed DE gene list of each study raises several problems that need to be kept in mind: different statistical methods and cutoff criteria (fold change and p-value)

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were used and not all genes were examined in all studies because of the use of various technologies (i.e., microarray platforms) resulting in lists with varying length.

Incomplete ranking in sense of lists of differentially expressed genes instead of all examined genes on the respective platform can be taken into account in the algorithm of the RRA method [63]. Since some genes were not measured in other platforms, they were not reported in multiple studies because they are not biologically relevant, but because they could not be measured at all in other studies. Hence, the compiled list of multi-study DEG due to titanium exposure may miss potential biological relevant DEG. This could also be the case, as the significance cutoff of $FC \geq 1.5$ and $p < 0.05$ to identify genes shared between studies could be too stringent [123]. However, this was due to the studies providing only pre-analyzed DEG lists to establish similar settings between studies. Since a consistent direction of expression change was only assumed when a gene was up-/downregulated equally in all studies or experiments, a limitation of potentially relevant multi-study genes is possible. The inconsistency in direction of gene expression may be based on the heterogeneity of the studies. For example, one transcriptome study [82] examined titanium exposure over a considerably longer period (6 days) than the others (at least 24 hours). Thus, if the variation is reflected in only one experiment compared to several others, it may be beneficial to include those in the list of multi-study genes as well. This would be the case, for example, for the gene CXCL2, as it is upregulated in three studies but downregulated in the long-term exposure study. Despite this and the variability among studies, most genes (74%) consistently reported between studies/experiments examining titanium exposure on cells were differentially expressed in the same direction. Hence, the common genes of the studies shown in the Supplemental Material (Table S1) could provide insights into the cellular response to titanium but have to be verified in additional studies [17,51].

Although only a few genes and proteins were common across different cell lines within a study exposing cells to titanium particles, similar changes in biological processes could be detected [84]. Since the number of DEG is usually very high, but different genes are highly correlated and act on similar biological processes, this

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discrepancy is expected to be smaller when genes are mapped to GO terms and pathways [124]. Another study indicates that despite the differences between the various microarray platforms and the small sample size, GO enrichment analysis yielded remarkably similar results between platforms and detected most of the relevant GO categories enriched in a reference list with larger sample size [108].

Hence, changes in biological processes and signaling pathways induced by titanium are discussed in the following. Common categories in GO and pathway enrichment analyses of proteome and transcriptome studies examining the cellular effects of titanium particles are: immune/inflammatory response, cell cycle regulation/apoptosis, stress response, and metabolism.

3.1.3 Impact of Titanium Particle Exposure on Cells

3.1.3.1 Transcriptome and Proteome Studies

Immune/Inflammatory Response Titanium particles – as foreign bodies – have the potential to amplify the immune response alongside triggering inflammation [17,125]. According to pathway enrichment analyses conducted on transcriptome and proteome studies, it appears that cells recognized titanium particles, potentially through pattern recognition receptors (‘Toll-like receptor signaling’, ‘NOD-like receptor signaling’), subsequently phagocytose or internalize them (‘Phagocytosis’, ‘Vesicles/endocytosis’), ultimately inducing an innate immune response (‘Innate immune system’, ‘Interferon signaling’, ‘Neutrophil activation’) [17]. During innate immunity, pattern recognition receptors (PRR) like toll-like receptors (TLR) recognize various ‘foreign’ patterns such as bacterial components like lipopolysaccharide (LPS) [126]. However, several *in vitro* studies [126–128] have confirmed the role of TLR in the cellular uptake of titanium particles and its involvement in the innate immune response triggered by titanium [17]. For instance, the stimulation of TLR4 by titanium initiated inflammatory signaling through the activation of NF- κ B [17,129]. The identification of various PRR, including NOD2, in proximity to aseptic loosening implants, coupled with the heightened expression of NOD2, the NF- κ B pathway, the MAPK pathway and the proinflammatory cytokine TNF- α

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in titanium-stimulated mice macrophages, implies a potential role of PRRs in wear particle-induced osteolysis [17,130].

The innate immune response in sense of a foreign body reaction possibly starts with the complement system and is controlled by cytokines [125], which is consistent with the results of the transcriptome studies. Increased expression of cytokines like interleukins and TNF- α in DEG and GO/pathway enrichment analyses was observed [17], whereas pro-inflammatory (e.g., IL1A, IL1B, IL8) and anti-inflammatory (e.g., IL10 and IL10-receptor, IL6-receptor) ones were affected. Titanium-induced inflammation was proven in many other *in vitro* studies using cytokine assays [39–42]. Furthermore, increased mRNA expression levels of chemokines were found in multiple transcriptome studies, such as CXCL2, CXCL3, CXCL8, CXCL20. Chemokines are produced, for example, by macrophages in response to particulate foreign bodies to attract leukocytes (chemotaxis), thus involved in inflammatory and immune response via G-protein receptors [131]. Among various chemokines, CXCL8 – also known as Interleukine-8 – is particularly noteworthy for its consistent upregulation across the majority of transcriptome analyses (4 studies with 6 experiments) [17]. Interleukin-8, associated with the early response to implant debris, is additionally regulated through the MAPK signal transduction pathway [17,131], which occurred often in the pathway enrichment analyses of selected *in vitro* and *in vivo* studies. MAPKs participate in a range of cellular reactions triggered by different stimuli, such as oxidative/genotoxic stress, proinflammatory cytokines or bacterial components like LPS; modifications in this pathway are linked to numerous human diseases [17,132]. In laboratory experiments with osteoblasts, the presence of titanium particles and ions led to the activation of MAP kinase members and subsequently to the activation of the IL-8 gene promoter via the NF- κ B pathway; this indicates that MAPK signal transduction plays a role in the chemokine response to titanium [17,133].

Cell Cycle Regulation and Apoptosis To repair the damage caused in the host tissue during titanium particle induced inflammation/stress, cell cycle arrest and even apoptosis may occur in the cells [134]. In general, changes in cell cycle

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or apoptotic processes were observed in both transcriptome and proteome studies, indicating another important process in cellular response to titanium. A signaling network of differentially expressed genes and proteins related to apoptosis and DNA repair caused by titanium exposure to macrophages revealed NF- κ B as central, thus connecting inflammatory and apoptotic processes [84]. Even if apoptotic cells are not always measurable, evidence of changes in the cell cycle or onset of apoptotic processes in proteomics/transcriptomics data can be found [78,81,135]. For example, an early stage of apoptosis could be reflected in alteration of cytoskeletal proteins since apoptosis is associated with drastic changes in cell morphology, as shown by a proteome study on lung cells [136]. Proteins of the cytoskeleton such as actin or tubulin (i.e., TUBA1B, ACTB) were also altered in both proteome studies included in this systematic review.

Various apoptotic pathways have been linked to titanium in the existing literature. Using a phosphoproteomic approach and long-term exposure to TiO₂ of up to two months, titanium was shown to modulate the phosphorylation levels of several proteins regulated by p53, and TP53 itself was enhanced phosphorylated and acetylated [137,138]. Hence, titanium-induced apoptosis via the p53 pathway might be at least partly impacted by dysregulated phosphorylation [137]. Additionally, epigenetic changes could play a role in this, as epigenetic analysis showed altered promoter methylation status of CDKN1A and SCARA3 in all tested cell lines (colon, liver, lung and skin cells) [139]. CDKN1A is regulated by TP53 linked to cell cycle regulation/apoptosis and SCARA3 is a cellular stress response gene by scavenging reactive oxidative species (ROS) [139]. Nevertheless, oxidative stress triggered by TiO₂ nanoparticles through increase of ROS did not activate the p53 signaling pathway in another *in vitro* study in human oral buccal epithelial cells, suggesting an alternative apoptotic mechanism [140]. For instance, lipid peroxidation through ROS production lead to lysosomal membrane destabilization and release of cathepsin B, which subsequently activates caspases and apoptosis [140]. This apoptotic pathway was evident in a study with bronchial epithelial cells exposed to titanium nanoparticles [141]. Consistently, cathepsin B was also

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upregulated in one selected proteome study [78]. In contrast to these intrinsic apoptotic pathways, extrinsic ones like death receptor pathways including Fas cell surface death receptor or member of TNF receptor superfamily binding TNF- α were less frequently reported in the context of titanium exposure [142].

Stress Response Cellular stress response is a defensive reaction to a strain on macromolecules caused by environmental influences such as inflammation resulting in deformation of proteins and damage to DNA, up to cell cycle arrest and programmed cell death [143]. Proteome and transcriptome analyses in one study pointed to a link between titanium-induced oxidative stress and inflammation (TNF- α) [17,84]. In addition, stress response was a common category in the functional enrichment assessments of transcriptome studies (see Figure 2.8) [17]. One quasi-universal characteristic of stress response is the increased level of ROS in cells [17,143]. Hence, multiple *in vitro* studies revealed that exposure to titanium particles led to an elevation in the production of ROS and oxidative products. These studies also noted an increase in the activity of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase [17,144–148]. For example, altered metabolism of glutathione, likely due to excessive ROS formation, was seen in two independent proteome studies by downregulation of Glutathione S-transferase P (GSTP1) [83,84]. This stimulation of antioxidant and detoxification enzymes represents the lowest level of oxidative stress [149]. It is followed by inflammation via MAPK and NF- κ B signaling pathways and apoptosis through mitochondrial perturbation as highest level [149], which was also observed in context of titanium.

Nevertheless, studies reporting no oxidative stress response due to titanium are also present in the literature; for example, Kocbeck et al. detected no generation of ROS after long-term treatment (3 months) with titanium nanoparticles in keratinocytes [150]. These partly contradictory results of *in vitro* studies on oxidative stress associated with titanium could be explained by the fact that titanium particles varying in size and crystal phase showed differential effects on ROS response [102]. Moreover, stress response depends on the expressed proteome and is thus cell

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type-dependent [143]. Although enhanced levels of ROS were not detected in other experiments of one proteome study, proteins mainly from the peroxiredoxin (PRDX1, PRDX2), superoxide dismutase (SOD2), or annexin (ANXA1, ANXA11, ANXA2, ANXA5) families, which are involved in the cellular response to stress, were impaired after TiO₂ exposure [17,78]. Titanium-induced altered expression (mostly downregulation) of peroxiredoxin – a family of antioxidant enzymes – was also seen in several other proteome studies using different cell lines and exposure times of up to two months (PRDX6, PRDX4, PRDX1, PRDX5) [83,135,136,138,151]. This also applies to proteins of the annexin (ANXA4, ANXA1, ANXA2, ANXA5, ANXA7) [83,136,138,151–153] and superoxide dismutase family (SOD1, SOD2) [83,135,136]. Hence, proteomics approaches can reveal a cellular stress response triggered by external stimuli like titanium particles, even if it cannot be detected by other methods such as intracellular ROS measurement.

Metabolic Processes GO and Pathway enrichment analysis of transcriptome and proteome studies revealed an impact of titanium particles on the metabolism of different cells. In particular, protein, lipid, carbohydrate and amino acid metabolism were affected.

Another proteome study in colon cells also showed many altered proteins involved in glucose metabolisms as well as energy homeostasis, suggesting that titanium affects the oxidative phosphorylation rate and ATP levels [136]. Glycolysis was further impaired by titanium nano- and microparticles in a transcriptome study in colon cancer cells with most of the genes involved being downregulated, indicating slowed or decreased production of pyruvate from this pathway [102]. In addition, many protein synthesis and degradation (protein ubiquitination) related genes were altered by various titanium particles but in an inconsistent pattern [102].

Similar results were also found in several metabolomics studies [154–157]. Jin et al. showed that in mouse fibroblast cells, TiO₂ nanoparticles suppressed the carbohydrate metabolism involving main pathways of cellular energy production such as the tricarboxylic acids cycle (TCA cycle), which could explain the observed serious

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damage to energy metabolism in mitochondria by titanium [155]. Furthermore, the reported decrease of ribose-5-phosphate implicated inhibition of the pentose phosphate pathway for entry to glycolysis, which normally generates NADPH [155]. This increased cellular oxidative stress as NADPH is essential for antioxidants like glutathione (GSH) [155]. Decreased cellular level of GSH through titanium exposure were differentially explained in another metabolomic study [156]. Therefore, in a human gingivitis model (human gingival fibroblasts + IL-1 β), TiO₂-NP reduced amino acids (e.g., cysteine, glutamic acid and glycine), which are important for the synthesis of GSH and thus favouring the oxidative state of cells [156]. Disturbed amino acids as well as disorder of purine and pyrimidine metabolism, suggesting an inhibition of DNA and RNA synthesis, was also demonstrated by another metabolomic analysis of mouse fibroblast cells [154]. In a multi-omics study using mouse macrophages, proteomic analysis indicated that mitochondrial membranes and functions were affected by TiO₂ nanoparticles as reflected in a metabolic approach by decreased ATP levels and downregulated metabolism in the TCA cycle [157]. This mitochondrial dysfunction was also evident in lipidomic analyses [157]. Thereby, most phospholipids from the cardiolipin class, which are primarily found in the inner membrane of mitochondria, were significantly downregulated in association with titanium exposure, possibly leading to an increase in mitochondrial ROS generation [157]. Using diverse omics approaches, the observed changes in the lipid, carbohydrate and amino acid metabolism caused by titanium particles potentially favour the generation of cellular oxidative stress.

3.1.3.2 Epigenome studies

Epigenetic changes are heritable or environmental stress-induced alterations in gene expression that are independent of modifications to the primary DNA sequence [158,159]. In addition to small and long non-coding RNA, other examples for the epigenetic regulatory mechanisms are DNA methylation and hydroxymethylation, histone modification, chromatin remodeling and RNA methylation [160].

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MicroRNAs (miRNAs) are a class of 21-25-nucleotide small non-coding RNAs, that regulate gene expression post-transcriptionally in a sequence-specific manner via translational repression and destabilization of their target gene [161]. Besides playing an important role in various cellular activities including cell growth, differentiation, development, and apoptosis, miRNAs are associated with diverse diseases such as cancer, cardiovascular or metabolic disease [162]. Several studies in plants, animals, and human cells indicated a significantly change in the expression of miRNAs associated with titanium, which affected biological processes such as development, autophagy, bone remodeling, inflammation and immune response [163–169].

Since the expression profile of miRNAs and their target genes is specific for different cell types [170], the effect of titanium on non-coding RNA was investigated only in oral related cells in this systematic review to be able to infer an association with peri-implantitis. Only one study that met these inclusion criteria was found [87]. Most of the reported miRNAs that were altered in macrophages after stimulation by titanium particles were members of the let-7 family, and almost all were downregulated [87].

Let-7 was discovered as the first human miRNA [162] and is one of the largest miRNA families highly conserved across species [171,172]. Thus, this alone could explain the high occurrence of these miRNAs. According to existing literature, let-7 plays a role in normal cellular development, but also in human cancer as tumor suppressor [172,173]. For example, Thai et al. indicated that the let-7/KRAS signaling pathway is altered by nano-TiO₂ treatment in lung epithelial cells, which is related to lung tumorigenesis [174]. However, the extent to which titanium particles promote cancer development in cells by altering miRNA expression is far from well understood.

Ndika et al. also showed a correlation of metal nanoparticles and seven miRNAs (miRs-142-5p, -142-5p*, -342-3p, -5100, -6087, -6894-3p and -7704) and its target genes were involved in biological processes linked to cellular response to metal ions and inflammatory response [87]. However, this miRNA-gene cluster was weakly correlated to titanium-NP, but stronger associated to ZnO and Ag nanoparticle

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exposure [87]. Since two of the seven miRNAs (miR-142, miR-342) were also differentially expressed in animal studies comparing inflamed to healthy peri-implant tissue, this might confirm their role in response to metal ion and inflammation in peri-implantitis.

Interestingly, different epigenetic mechanism can influence each other. For instance, DNA methylation modifies miRNAs or miRNAs regulate epigenetic modifiers such as DNA methyltransferases (DNMTs) or histone deacetylases [162].

Among the available studies, only the effect of titanium particles on global DNA methylation and hydroxymethylation, which play key roles in epigenetic silencing of transcription, has been investigated [158]. Covalent binding of a methyl group at the C5 position of cytosines, catalyzed by DNA methyltransferases, affects the accessibility of DNA to the cellular transcriptional machinery [175]. 5-hydroxymethylcytosine (5hmC), an oxidized form of 5-methylcytosine (5mC), is considered to be key intermediate in active demethylation pathways [176]; but no effect on the 5hmC level in oral cells as well as in lung cells exposed to TiO₂-NP was detected [88,177]. However, it has been investigated only in few studies by now, requiring further research in this topic.

In contrast, a decrease in the 5mC level in the sense of a global hypomethylation by TiO₂-NP was observed in human macrophages by one study, although this is only verified in a downregulation of DNA methyltransferases in another study [88,89]. Global DNA hypomethylation has been associated with genomic instability, altered gene expression, elevated DNA damage, as well as several diseases and cancer [178]. Because global hypomethylation was detected even at non-cytotoxic concentrations [89], epigenetic changes might be an early effect of cellular response to TiO₂-NP. The precise causes behind demethylation remain uncertain, yet the absence of DNA methyltransferase activity is associated with genomic hypomethylation [17,178]. This loss of activity might result from heightened oxidative stress, as the production pathways of antioxidant proteins such as GSH are linked to S-adenosylmethionine biosynthesis – a crucial co-factor for methyltransferases [17,179]. Interestingly, both methionine and S-adenosylmethionine were significantly reduced after exposure

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to IL-1 β /TiO₂ in an *in vitro* metabolomic study using a human gingivitis model [156]. Therefore, the decreased gene expression of several DNA methyltransferases observed by Lu et al. and the downregulation of multiple methylation-related genes/proteins in other studies in lung, liver or skin cells may induce global genomic hypomethylation as a result of inhibitory effects on the DNA methylation process by titanium particles [88,139,148]. However, no consistent downregulation of DNA methyltransferases by titanium particles could be shown in all experiments and differed in the cell lines [88,139,148].

The conflicting results regarding the alteration of 5mC levels by titanium in the two studies selected in this systematic review (no alteration and global DNA hypomethylation) can be attributed to the different study design (particle type, cell line) as well as the methods used: liquid chromatography tandem mass spectrometry (LC-MS/MS) and enzyme immunoassay. Since the later method has limits in terms of reproducibility and reliability, and measures the relative 5mC content indirectly, LC-MS/MS should be used in further studies because of its high sensitive, reproducible and direct quantitative measurement [180].

Cumulatively unchanged DNA methylation levels can also be a result of global DNA methylation approaches by masking hypomethylation of one and hypermethylation of other genomic loci [88,148]. However, studies additionally investigating promoter methylation status of selected genes or identifying differentially methylated sequences by microarray-based profiling revealed only little effect on the epigenome of human lung, liver or skin cells after (long-term) exposure with titanium nanoparticles [139,160].

Because of the limited scope of studies and the partly contradictory results, definitive conclusions on the epigenetic impact of titanium on cells are not feasible [17]. In addition, studies investigating the influence of titanium on other epigenetic mechanism such as histone modifications are needed.

3.1.4 Impact of Titanium Ions Exposure on Cells

Besides metallic debris, titanium ions are also released in the surrounding tissue of implants, especially once the titanium oxide layer's integrity is impaired by an acidic environment [17,181]. One PECO question therefore concerned the cellular effects of titanium ions [17] at the transcriptome, proteome, or epigenome level as there are conflicting results in the literature on the impact of titanium ions on cellular functions. However, no omics study was found on this topic [17].

Two separate studies noted a significant increase in toll-like receptor 4 (TLR4) gene and protein expression in gingival epithelial cells or tissue exposed to 9 ppm of titanium ions compared to those not exposed to titanium [17,43,182]. TLR4 and TLR9 that recognize bacteria were also upregulated in the interface membrane around loosening total hip replacement implants, but downregulated after stimulation with titanium particles, possibly to prevent excessive and harmful reactions of the host [183]. Consistent with the omics studies reviewed, further research showed that titanium particles can directly attach to TLR4 without the LPS protein complex, triggering an inflammatory response [17,127,129]. This suggests that both titanium ions and particles interfere with the Toll-like receptor signaling pathway [17]. Another study on a Co-Cr-Mo alloy also suggested that both soluble and particulate metal implant debris initiate a proinflammatory response in monocytes/macrophages [184]. Inflammatory reaction and oxidative stress caused by titanium ions (1-5 ppm) was also shown in another *in vitro* study with macrophages by increasing the TNF- α and SOD (a scavenger of superoxid) secretion [185]. On the other hand, the release of TNF- α from neutrophils was elevated only by 1-3 μm titanium particles, but not by Ti ions (10 ppm) in another study [186]. Furthermore, titanium ions act as inhibitor of the differentiation of osteoblasts [187,188] as well as inducer of osteoclast differentiation [189], suggesting to play a role in bone resorption. However, a decrease in resorption ability of osteoclasts was also observed under Ti ion exposure in the same study [189] and confirmed in another study [190].

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Several other studies showed no effect of titanium ions on the expression of certain genes such as CCL2, IL-1 β , RANKL, OPG and cytokine secretion, but Ti ions enhanced the expression in cells primed with LPS (see section 3.1.6) [41,43,182]. Pettersson et al. used filtration (0.22 μ m) to examine the distinct effect of titanium ions and complexes/particles formed in the culture medium [41]. They indicated that the stimulation of IL-1 β secretion of LPS-primed monocytes (THP-1) and associated inflammatory response is caused by particles, whereas the cytotoxicity is triggered by free soluble titanium ions [41]. The cytotoxic effect of titanium ions with concentrations above 10 ppm was proven in further studies [43,182,186,187].

Cellular responses to metal ions and particles could vary due to different cellular uptake mechanisms – while ions cannot easily cross the cell membrane, (nano)particles can penetrate through pino-/endocytosis pathways [17,191]. Therefore, Poon et al. showed that particles with low solubility, in particular TiO₂ and Ag, achieved higher cellular concentration than the more soluble ZnO particles and silver ions [90]. The lower number of ion-associated genes triggered by silver ions reported in a transcriptome study also reflects the limited intracellular accessibility to free ions [85]. On the other hand, soluble particles can release ions inside the cells [191], which make it difficult to study the differences between metal ions and particles in cellular response. Nevertheless, to differentiate the specific impacts of titanium ions or particles on cells and their underlying mechanisms, more comparative studies are required, incorporating omics approaches [17]. Two studies involving silver and zinc oxide ions/particles have already demonstrated the difference in cellular effects between metal particles and ions at the transcriptome level [17,85,91]. Another study using global transcriptomics suggested that the cellular response to ZnO-NP is due to leached Zn²⁺ ions [86]. Furthermore, one *in vitro* study concluded that silver nanoparticles – not ions – induce global epigenetic changes in sense of hypermethylation in different cell lines [192].

3.1.5 Impact of Various Metal Exposure on Cells

Alongside the predominant element titanium, vanadium and aluminum are also components of the metal debris in the peri-implant tissue, resulting from corrosion of the titanium alloy Ti₆Al₄V used in dental implants [17,193,194]. Additional metallic elements such as iron, zinc, magnesium, or nickel were found within the soft tissue affected by peri-implantitis [17,195], as well as chromium, cobalt or molybdenum due to dental prostheses such as CoCr abutments [193]. Consequently, PECO question 1c was designed to establish whether titanium elicits similar or unique cellular effects compared to other metals at the transcriptome, proteome or epigenome level [17].

Merely six percent of all DE miRNAs were identical among cells treated with TiO₂, Ag, and ZnO [17] and no common set of genes was found to be modulated by TiO₂, Fe₂O₃, and ZnO nanoparticles in colon-derived cells [87,91], implying specific gene expression patterns for various metal types [17]. However, common biological processes between metal particles were observed in the transcriptome studies. Genes related to the family of metallothioneins were upregulated in multiple cell lines and various metal particles and ions [85,86]. Metallothioneins are metal-binding proteins involved in homeostatic control of essential metals and detoxification by reducing the presence of metals in the cytoplasm [196]. These proteins are induced by a wide range of stimuli, naturally by metals such as cadmium and zinc, but also by ROS [196,197]. An association of titanium with metallothioneins is rarely reported in the literature; moreover, transcriptome studies found, if any, only a slight upregulation of few metallothioneins in cells with short-time exposure to titanium [79,83]. However, one study stimulating macrophages for six days with titanium particles detected enhanced gene expression levels of several metallothioneins [82]. This indicates that these metal-binding proteins play a more important role in the initial cellular response to metals such as ZnO and Ag than TiO₂.

Nevertheless, the cellular inflammatory or immune response induced by TiO₂, as observed in several transcriptome studies (see section 3.1.3), appears comparable to that of other metals [17], although in these comparative studies no DEG by

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TiO₂ were found. Thus, increased gene expression of various chemokines, TNF- α , and IL-1 β was observed in distinct cells following exposure to ZnO and Ag [17,85,86]. Furthermore, cell exposure to ZnO and Ag particles induced pattern recognition via TLR, resulting in inflammasome activation [85], akin to previous observations in cells stimulated by titanium [17]. Another *in vitro* study using THP-1 cells revealed that diverse metal nanoparticles can elevate the gene expression of TLRs (i.e. TLR4 and TLR6), albeit with varying degrees depending on the specific metal type [17,198]. ‘Unfolded protein response’ was a highly enriched process in cells exposed to ZnO and Ag particles [85,86], but was only present in one titanium exposure study. Nanoparticles can bind to proteins and cause conformational changes that could induce an immune response by exposing normally buried sequences as well as inflammation or apoptosis [199]. However, this is particle type dependent [199], which might explain the observed difference. For example, titanium seems to interact with phosphate groups in phosphorylated proteins and phospholipids as reflected by a more pronounced alteration of the phosphoproteome than of the whole proteome which distinguishes titanium from other metal particles [157]. Further metal specific cellular responses were demonstrated in several *in vitro* experiments [200–203], as well as common processes such as DNA damage, ROS generation and apoptosis, but with different extent depending on the type of metal, especially concerning the genotoxicity [204–207].

As mentioned above, titanium is not the only metal in peri-implant tissue, so it should be taken into account that a mixture of metal particles/ions may affect the cellular response in another way than one metal type alone. Evidence for this was provided in experiments on two different human cell lines, where titanium nanoparticles potentiated the cytotoxicity, oxidative stress and apoptosis response of cadmium [208]. Furthermore, an *in vivo* study demonstrated that TiO₂-NP increase the uptake and toxicity of other metals (Cd and Zn) in guts of animals [209]. This potentially synergistic effect of metals has to be further investigated.

In addition, more comparative studies should be planned to examine the different effect of titanium and other metals on the epigenome, as only a few were available

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to date. No effect on the global DNA methylation by titanium or copper oxide was observed in oral cells [88]. One study with other cell lines demonstrated an increase in 5mC-levels due to silver nanoparticles, but not to TiO₂, Au or ZnO [192]. In contrast, global hypomethylation (compared to a positive control) was seen in another study on lung fibroblasts stimulated with ZnO and TiO₂ nanoparticles [210]. Since only the changes in DNA methylation induced by titanium compared to other metals were studied, further studies on changes in other epigenetic mechanisms are needed to draw conclusions. For example, histone modifications by metals such as chromium, nickel and arsenic have already been detected [211].

3.1.6 The Synergistic Effect of Titanium and Bacteria on Cells

The oral environment leads to a continual interaction of dental implants with bacteria, facilitating the formation of biofilms on these implants [17,212]. Bacterial components such as lipopolysaccharide (LPS) of the Gram-negative bacterial membrane – the classic endotoxin – but also peptidoglycan (PG) and lipoteichoic acids (LTA) from Gram-positive bacteria can be detected through immune cells as pathogen-associated molecular patterns (PAMPs) triggering inflammation [17,213]. The endotoxins found in the peri-implant tissue may be caused from biofilms on implants. Titanium wear particles, however, also have a high affinity for binding systemic endotoxins [17,214]. Therefore, the joint effect of titanium implant wear and bacteria on the cellular response was examined in PECO question 1d [17].

Regarding this query, no omics study was identified, but various *in vitro* experiments provide insights [17]. As reviewed by Lieder et al., multiple studies similarly concluded that titanium particles contaminated with endotoxin induce greater cytokine secretion, increase osteoclast differentiation, and decrease implant osseointegration than titanium particles alone [214]. For instance, a synergistic effect of titanium ions or particles and LPS on the enrichment of chemokine ligand 2 (CCL2), TNF- α , IL-1 β , IL6, or IL8, along with an increased RANKL/OPG ratio, was detected in human and mice cells [17,41,43,182,215,216]. In a probably more

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realistic *in vitro* study, peri-implant granulation tissue fibroblasts from ten patients with peri-implantitis were exposed to TiO₂ particles and viable *P. gingivalis*, not only LPS [217]. This revealed increased expression of pro-inflammatory mediators by titanium and bacteria alone, but also synergistically enhanced expression of TNF- α at both gene and protein levels by combined exposure of TiO₂ particles and *P. gingivalis* [217]. To gain a more precise idea of immune/inflammatory processes, one study examined the alteration of 205 allergy and inflammation related genes via DNA allergy chip in titanium and/or LPS-stimulated human macrophages [218]. Upregulation of 17 inflammation related genes (with more than twofold change) occurred independently and synergistically by titanium particles and LPS, whereas dual stimulation most upregulated three genes (IL1B, IL6 and IL8) [218]. In those studies, it was hypothesized that titanium enhances the sensitivity of the epithelium to oral bacteria by increasing the expression of TLR4 or by acting as a second stimulus of LPS to activate and release IL-1 β , thereby exacerbating inflammation [17,41,43,217,218]. Islam et al. suggested that particulate-bound endotoxins (LPS and LTA) activate toll-like receptors by different mechanisms than soluble ones [219].

However, the exact mechanism is not yet completely understood, so omics studies may shed light on this issue. One transcriptome study was found examining the effect of co-treatment of TNF- α and metal nanoparticles (TiO₂, ZnO, SiO₂, and Fe₂O₃) to imitate a pre-existing inflammatory state in colon cell lines using microarray expression analysis [91]. Since no change in gene transcription response and cell viability was detected compared to isolated exposure of metals after four hours, the authors concluded that inflammation does not enhance the response to nanoparticles [91]. In contrast, another study culturing mesenchymal stem cells with TiO₂-NP and TNF- α up to 21 days showed a decrease in genes involved in osteogenesis and adipogenesis caused by titanium and inflammatory conditions alone, which was enhanced by co-stimulation [220].

To prove these *in vitro* results, further *in vivo* experiments are necessary. For example, Wachi et al. injected LPS and titanium ions separately and together in the gingiva of 8-week-old Wistar rats [17,43]. The combined exposure (LPS and Ti

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ions) led to elevated mRNA expression of CCL2 and an increased RANKL/OPG ratio compared to single stimulation [17,43].

3.1.7 Differences of Healthy Dental Implants and Teeth

The PICO questions 2/3a were designed to examine the differences in cell expression patterns within the tissue surrounding implants and teeth under similar environmental conditions, regardless of the presence of inflammation [51].

As the removal of tissue from healthy implants or teeth is ethically questionable, this study design was not used in human studies, but in animal studies [51]. However, there are possibilities such as sampling from extracted wisdom teeth or tissue excision for contour site development around implants as shown in one discovery cohort study [96].

Only two studies in total were available [92,93]. Because of the selectively reporting of DEG (only 39 up- or downregulated DEG were stated), a pattern of common DEG in all studies is not ascertainable, although the microarray analyses, animal model and follow-up used in these studies would be comparable. As we did not receive a response from the authors to the request of the availability of these data and none of the studies carried out GO or pathway enrichment analyses, a more profound analysis could not be performed.

Reasons for different gene expression in peri-implant soft tissue relative to controls can only be conjectured since no additional experiments were carried out in those animal studies [51]. Nonetheless, certain patterns in cellular expression were similar to the cellular response to titanium indicated by in vitro studies of PICO1a [51]. For example, genes associated with inflammatory and immune responses, like chemokines (CXCL2, CCL2), were also enhanced in trials exposing human microdermal endothelial cells and macrophages to titanium [51,79,83]. The upregulation of inflammatory markers in peri-implant tissue was also confirmed in another animal study on rats via RT-PCR, whereas CXCL2 was found in both the epithelium and connective tissue [221]. In addition, Kobayashi et al. revealed a significant increase in the expression of Lipopolysaccharide (LPS) binding protein

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(LBP) in peri-implant tissue in comparison to both periodontal and oral mucosal connective tissue [51]. Functioning as a soluble acute-phase protein, LBP has a strong affinity for binding to LPS and subsequently transfers it to CD14 for recognition by the TLR4–MD-2 complex, thereby amplifying the effects of endotoxins [51,222,223]. Considering the findings from cellular studies indicating the binding of titanium particles to LPS, the upregulation of LPS-binding TLR4 expression under titanium exposure, and the synergistic enhancement of the cellular immune/inflammatory response by LPS and titanium (see section 3.1.3), it is plausible that the increased expression of LBP in healthy peri-implant tissues, in contrast to tissues with no implant, may be attributed to the presence of the titanium implant material [51].

Furthermore, the G0/G1 switch 2 gene was found to be upregulated in both peri-implant rat tissue and titanium exposed M1 macrophages [79,92]. In another study using microarray analysis, stimulation of human fibroblasts with TNF- α revealed G0S2 as the most upregulated gene mediated by NF- κ B pathway and promote apoptosis by inhibiting the anti-apoptotic protein Bcl-2 [224]. Thus, as indicated by *in vitro* studies, titanium and the associated stimulation of TNF- α secretion could promote apoptosis partly via upregulation of the G0/G1 switch 2 gene in peri-implant tissue. Another gene (FOS) which is associated with apoptotic processes was shown to be upregulated in studies regarding PICO 1 and 2. Moreover, the c-Fos gene is related to osteoclast differentiation and its gene and protein expression was enhanced by titanium particles in mice macrophages [225].

The results of the animal studies also pointed to heightened oxidative stress in the peri-implant tissue relative to healthy controls due to changes in the expression of genes linked to the neutralization of reactive oxygen species [51,92,93]. However, in a cross-sectional study, no significant ROS levels were found in ten samples of healthy peri-implant tissue, while no Ti particles were present either [220]. In contrast, the production of ROS was observed simultaneously alongside the occurrence of titanium particles in 60 peri-implantitis samples [51,220]. This, in combination with the results of the *in vitro* studies, supports the assumption that titanium particles in the peri-implant tissue can lead to oxidative stress in cells.

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Furthermore, it is noteworthy that the sampling was performed four weeks after implantation and factors that cause implant debris such as corrosion by fluoride from tooth paste or micro-movements through occlusal loading during mastication [29] were not incorporated in the animal model. Hence, it is questionable whether titanium particles were present in sufficient quantity in the surrounding tissue to cause cellular changes. On the other hand, it has been proven that particle wear is already possible during implant placement [181]. Additionally, the possibility of titanium particles being released from healthy dental implants into bone tissue was demonstrated in a post-mortem study involving a total of ten people [33]. To examine whether the difference in cellular response in healthy tissue surrounding dental implants or no implants is based on the implant material (titanium) itself, more well designed *in vivo* studies with appropriate statistical methods and additional analyses of the titanium particle content in peri-implant tissue are needed.

3.1.8 Differences of Peri-Implantitis and Healthy Implants

Questions 2b and 3b, respectively, were conducted to determine similarities and differences in tissues around implants with peri-implantitis and healthy implants in both animal and human subjects with the aim of revealing the processes that promote peri-implantitis at different molecular levels [51].

Only one of the two animal studies reported a list of DE miRNAs, so no comparison was possible. In addition, the statistical test to find DE miRNAs between test and control group was not described in either study, complicating the reproducibility of the results and increasing the risk of bias. However, the validation of selected miRNAs by RT-PCR revealed similar results in the two *in vivo* studies. Namely, a downregulation of let-7g and miR-27a and an upregulation of miR-145 [94,95].

One study further investigated the effects of miR-27a on canine bone marrow stromal cells; it was found that osteogenesis and angiogenesis are promoted by reducing the inhibitory role of TNF- α by activating the Wnt signaling pathway

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[51,95]. Consequently, the decrease in miR-27a expression induced by TNF- α could impede bone formation in peri-implantitis [51,95]. Additionally, the changed expression levels of miR-145 and let-7g in peri-implantitis tissue were linked to osteoclastogenesis [226,227], implying the involvement of microRNAs in the regulation of bone remodeling in peri-implantitis [51]. A subsequent *in vivo* experiment also showed that a miR-27a-enhanced delivery system can improve bone regeneration and reosseointegration of peri-implantitis defects [95]. In addition, LPS stimulation of mice or human macrophages through TLR2 and TLR4 engagement led to downregulation of miR-27a [228]. This was associated with increased IL-10 expression to prevent overly exuberant inflammatory responses [228], underlining the role of miR-27a in peri-implantitis. Moreover, in one of the two reviewed animal studies [94], the pathway enrichment analysis demonstrated an upregulation of the toll-like receptor signaling pathway in peri-implantitis in comparison to healthy implants [51]. As indicated by the *in vitro* transcriptome studies (see section 3.1.3), titanium particles also influence the toll-like receptor signaling pathway and upregulate IL10 gene expression [79,81,229], suggesting TiO₂ together with bacterial components like LPS can alter the inflammatory response and bone formation in peri-implant tissue. Additionally, as also observed in titanium exposed cell studies, pathways like MAPK signaling pathway or NF- κ B signaling pathway were highly enriched in the KEGG analysis of target genes of altered miRNAs in peri-implantitis tissue [94]. This leads to the hypothesis that these altered signaling pathways in peri-implantitis are influenced by the implant material titanium.

However, the role of miRNAs in peri-implant tissue is rarely studied. Existing human studies have focused on the use of microRNAs as biomarkers for the diagnosis or prognosis of peri-implantitis rather than investigating their regulatory mechanisms. For example, Urvasizoglu et al. identified 179 miRNAs differentially expressed in saliva of peri-implantitis patients compared to healthy ones, but concentrated mainly on miR-4484 as potential biomarker for peri-implantitis [230]. On the other hand, pathway enrichment analysis in a study examining the expression of miRNAs in crevicular fluid of inflamed peri-miniscrew implants and periodontitis

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affected teeth revealed pathways related to inflammatory regulation and osteoclast activity in peri-implantitis lesions [231].

Only one human study with small sample size comparing healthy and inflamed peri-implant tissue at the transcriptome level met the inclusion criteria for PICO 3b. Thereby, smokers or patients with systemic diseases like diabetes were excluded to avoid additional confounding factors affecting cellular gene/protein expression between subjects. Remarkably, baseline levels of inflammation and oxidative stress were elevated in tissues around implants regardless of disease status [51,96]. This is consistent with the hypothesis that the implant material itself causes cellular changes resulting in increased immune/inflammatory responses and oxidative stress, as discussed previously (see section 3.1.3 and 3.1.7). Furthermore, genes involved in the endosomal-lysosomal pathway, like WASH1 or BLOC1S4, were highly upregulated in peri-implantitis tissue, presumably due to phagocytosis of titanium particles [96]. Since no measurement of the titanium content in the peri-implant tissue was performed, it can only be assumed that this change in gene expression is triggered by titanium. However, enrichment of cytoskeletal proteins and proteins associated with vesicular transport or endocytosis was also found in titanium-stimulated human macrophages, keratinocytes or microdermal endothelial cells [78,83,135].

Moreover, the study by Martin et al. revealed that genes associated with bacterial system response were not upregulated in peri-implantitis compared to healthy implants [96], implying that factors beyond bacterial infection may be involved in the inflammatory processes around implants [51]. Despite findings from a meta-analysis of 19 studies that demonstrated a heightened presence of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* in the plaque of inflamed implants compared to healthy ones, the majority of the 64 trials examined in the review by Daubert et al. did not consistently identify a specific microbial profile for peri-implantitis [51,212]. This points to another factor influencing the composition of the peri-implant microbiome [51]. Additionally, a human study comparing the transcriptome in peri-implantitis and periodontitis patient, which was excluded

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from this systematic review because of the inclusion of one smoker in the peri-implantitis group, revealed innate immune and defense response as dominating GO categories in inflamed peri-implant tissue [232]. In contrast, genes related to bacterial response system prevailed in periodontitis samples [232]. This provides evidence that inflammatory processes in the peri-implant tissue are not as strongly promoted by bacterial influences as in periodontitis, which allows scope for an additional trigger factor for peri-implantitis.

As discussed in PECO 1d, titanium particles and the bacteria in peri-implant tissue can have a mutual impact on cells [51]. In a laboratory setting, it was shown that microorganisms within a biofilm on a titanium dental implant exhibit electroactivity, resulting in spontaneous electricity production and corrosion of the titanium implant [51,233]. Such dissolution products of titanium in turn have the potential to alter the structure of the peri-implant microbiome and diminish its diversity [51,234]. To verify these correlations between titanium and changes in peri-implant tissues and microbiome favoring peri-implantitis, more trials with simultaneous investigation of titanium level in a sizeable cohort are required [51]. Thus, the following study by Daubert et al. is particularly noteworthy, as global DNA methylation levels were investigated along with titanium content in inflamed and healthy peri-implant tissues. Global DNA hypermethylation has been shown to correlate with higher titanium levels. Consequently, significantly higher 5mC levels were seen at implants with peri-implantitis than healthy ones [235], which could be explained by higher content of titanium particles in peri-implantitis tissue. This is confirmed in several other studies [34,35,220]. Nevertheless, the *in vitro* studies regarding PECO 1 showed global hypomethylation as cellular response to titanium exposure, if an epigenetic change was detectable, whereas silver nanoparticles increased the 5mC content in THP-1 cells [192]. So, although there is evidence that (titanium) implant debris can affect the epigenome in peri-implant tissues, the consequences of this need to be further explored.

3.1.9 Differences of Peri-Implantitis and Periodontitis

3.1.9.1 Transcriptome Studies

Since peri-implantitis and periodontitis show both clinical differences and similarities [14], PICO question 2c/3c was intended to investigate how these two oral diseases differ at the genome, epigenome, proteome, and transcriptome level.

Although no animal study addressed this issue, four human studies were included in this systematic review showing all distinct gene expression patterns at both mRNA and lnc/miRNA levels for peri-implantitis and periodontitis [51]. Furthermore, in studies examining healthy periodontal tissue as additional control group peri-implantitis specimens showed a greater discrepancy with the healthy state than periodontitis ones [75,99].

Although the immune cell composition is qualitatively similar in peri-implant to that in periodontal inflammation, peri-implantitis is marked by a more severe inflammatory infiltrate and innate immune response, as well as more severe and rapid tissue destruction [236]. Similar observations are evident in the transcriptome studies eligible for this systematic review.

Remarkably, immune response was seen predominant in peri-implantitis relative to periodontitis in almost all studies. For instance, GO terms related to the innate immune response were highly enriched in peri-implantitis compared to periodontitis as well as healthy tissue samples and DEG between this comparison were associated with cytokine secretion, among others [99]. Cytokine gene expression similar to that observed in the titanium exposure studies (PECO 1) was also observed by Bressan et al. [220]. They detected the chemokine CXCL2 along with other inflammatory markers (IL6, IL1B, IL10, PTGS2) in peri-implantitis samples from 60 patients in which the presence of titanium particles was concurrently verified [220]. This supports the hypothesis that discrepancies in the immune response found in transcriptome studies contrasting peri-implantitis and periodontitis may be linked to the foreign body reaction to titanium wear debris. Increased levels of chemotactic cytokines such as macrophage chemotactic protein-1 (CCL2) or Interleukine-8 were also found in the tissue of retrieved orthopedic implants with periprosthetic osteolysis

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and cells exposed to titanium alloy, thus playing a role in the immune response of peri-implant tissue to wear debris [131]. Since *in vitro* studies indicate that toll-like receptors are involved in the innate immune response induced by titanium [129] and their enhanced expression by titanium may increase the sensitivity of the epithelium to oral bacteria [41], the observed enrichment of toll-like or NOD-like receptors in GO and pathway enrichment analysis in almost all human studies comparing peri-implantitis with periodontitis may be due to the implant material [75,98,99].

The pathogenesis of peri-implantitis is strongly influenced by the immunological microenvironment as the immune response causes more extensive tissue damage than that caused by bacterial pathogens themselves [51,237]. For instance, the activation of inflammasomes, potentially triggered by titanium particles/ions, induces inflammatory bone resorption by enhancing the differentiation of osteoclasts through the interaction of RANK with its ligand RANKL [51,238]. As shown in a cross-sectional study comparing peri-implantitis and periodontitis, the RANKL/RANK/OPG profiles of these two inflammatory oral diseases differed, resulting in higher RANK concentrations in peri-implantitis samples, which could be explained by the immunological hyper-responsiveness in peri-implantitis [239]. In alignment with the reported accelerated bone resorption in peri-implantitis compared to periodontitis, a transcriptome study within this systematic review revealed an elevation in osteoclast differentiation-related pathways together with an increased RANKL/OPG ratio in peri-implantitis [17,51,75]. Thereby, RANKL expression appeared to be under lncRNA control [75]. In the same study, the cyclooxygenase pathway was the most prominent pathway upregulated in inflamed implants compared to inflamed teeth [75]. The inflammatory prostaglandin PGE2 – a product of cyclooxygenase (COX) – can stimulate bone resorption by altering osteoclast/osteoblast differentiation via increased expression of c-Fos and activation of RANKL [240]. Thus, increased COX-2 expression around aseptically loose orthopedic implants and titanium-induced COX-2 depended production of PGE2 in mice fibroblasts indicate that the cyclooxygenase pathway is involved in particle-stimulated osteolysis [241].

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Besides this inflammatory bone resorption, the excessive recruitment of neutrophils in peri-implant tissue may lead to the accumulation of matrix metalloproteinases (MMPs), causing the destruction of soft tissue [51]. Consistently, Bressan et al. showed an upregulation of several MMPs (MMP1, MMP2, MMP7, and MMP9) in inflamed peri-implant tissue as well as decreased expression of their inhibitor TIMP1 [51,220]. Differences in the transcripts of MMPs and other proteases such as cathepsin involved in extracellular matrix (ECM) degradation were observed in peri-implantitis relative to periodontitis samples; similar variations were found in cells exposed to titanium particles in selected studies of this systematic review [51]. For example, cathepsin K was upregulated in three transcriptome studies of PECO 1a [79,82,84] which is, along with MMPs, an important collagenolytic bone protease with a central role in aseptic loosening of implants in orthopedics [242].

MMPs not only regulate the inflammatory response by controlling chemokines, but also play a role in splitting components of cell-cell junctions and cell-matrix contacts while remodeling the ECM [51,243]. GO enrichment analysis showed a greater downregulation of genes associated with cell adhesion molecules, such as hemidesmosomes, in peri-implantitis compared to periodontitis soft tissue pointing to a weaker epithelial barrier at inflamed implants relative to teeth [51,75]. In the same study, titanium-associated microtubular dysfunction was found to be under lncRNA control in peri-implantitis tissue [75]. Additionally, an *in vitro* study indicated that titanium particles induce alterations in cytoskeletal proteins, leading to the disruption of endothelial cell-cell adhesion at adherens junctions in human microdermal endothelial cells [51,83]. Given the observed disruption of cell-cell interactions, including tight junctions and adherens junctions, by periodontal pathogens in periodontitis [244], the concurrent influence of titanium and bacteria in peri-implant tissues could potentially accelerate the rapid progression of peri-implantitis by modifying epithelial barrier function [17,51].

In addition to these factors, oxidative stress, represented, for example, by an increase in ROS, is a crucial factor in the destruction of both hard and soft tissue and has been associated with oral diseases, including periodontitis [51,245]. Aside

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from periodontal inflammation, titanium triggers an increased production of ROS in the oral cavity [17,245]. Accordingly, ROS production was found in 60 peri-implantitis specimens alongside the presence of titanium particles [51,220]. GO enrichment analysis of genes differentially expressed in peri-implantitis compared to periodontitis also showed GO terms linked to ROS response are more enriched in peri-implantitis tissue [99]. Furthermore, a transcriptome study of PICO 3b showed heightened baseline levels of inflammation and oxidative stress within tissue adjacent to implants, regardless of the disease status [51,96]. The results of animal studies selected for PICO 2a also pointed to enhanced oxidative stress in healthy peri-implant tissues in comparison to periodontal tissues through changes in the expression of genes related to the neutralization of ROS (SOD3, LPO) [51,92,93]. Hence, the significantly higher response to ROS and associated tissue destruction in peri-implantitis relative to periodontitis specimens could be explained by the supplementary impact of titanium on cellular stress in the peri-implant tissue [51].

3.1.9.2 Epigenome Studies

Environmental stress is one factor leading to epigenetic changes such as DNA methylation or non-coding RNAs [17,160] and may affect the onset and advancement of peri-implantitis.

Besides the classical RNA-RNA interaction of RNA transcripts and microRNAs (miRNAs, <200 nucleotides), long non-coding RNAs (lncRNAs) which comprise more than 200 nucleotides can act as competing endogenous RNAs (ceRNAs) like a sponge molecule of miRNAs and inhibit their effect on target genes [140]. Hence, the long non-coding transcriptome can impact diverse biological processes like immune response and playing a role in the pathogenesis of diseases like cancer [140]. The regulatory role of lncRNAs in periodontal inflammation was also evident in an *in vitro* study on human gingival fibroblasts showing enhanced proinflammatory cytokine production and expression of TLR4 stimulated by the lncRNA MALAT1 by sponging and thus inhibiting miR-20a [246]. Therefore, several human studies already investigated the lncRNA profile of periodontitis and established periodontitis

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associated ceRNA networks [247–249]. For example, Tang and colleagues integrated multiple datasets of microarray analysis comparing patients with periodontitis and healthy teeth resulting in a total of 522 samples to explore the lncRNA expression profile and its correlation to immunity in periodontitis [250]. A classification model validated internally and externally in another dataset revealed seven lncRNAs in periodontitis that were distinct from healthy samples and strongly correlated with immune signatures; in particular, the lncRNA MIAT and its ceRNA network of five miRNAs and 100 mRNAs appeared to be involved in several immunological processes, especially B cell activation, proliferation, and differentiation [250]. Interestingly, the miR-1246 found downregulated in this ceRNA network was significantly lower expressed in the top 20 DE miRNAs in a transcriptome study comparing peri-implantitis and periodontitis [99], providing a slight indication of a more intensive immune response in peri-implantitis.

In general, the influence of non-coding RNAs on the pathogenesis of peri-implantitis has been less researched. Due to selective reporting, the results of studies investigating differences in long non-coding RNA of peri-implantitis and periodontitis samples are not directly comparable with each other. As already mentioned previously, these studies indicated that some biological processes in peri-implantitis might be under lncRNA control such as bone resorption (RANKL expression), immune response or changes in cytoskeleton (microtubular dysfunction) and thus cell adhesion [75,99]. Moreover, an animal study eligible for PECO 2a [94] indicate that miRNAs are involved in bone resorption in peri-implantitis (see section 3.1.8). The immunogenomic landscape of peri-implantitis based on the interaction of lncRNAs, miRNAs and mRNAs was examined in an *in silico* study [237]. GO enrichment analysis of immune related genes in the ceRNA network revealed an involvement of inflammatory pathways [237]. Additionally, pathways such as ‘MAPK signaling pathway’, ‘Chemokine signaling pathway’ or ‘Osteoclast differentiation’ were enriched in the KEGG signaling pathway analysis [237]. Enrichment analysis of target genes of the ceRNA network of one transcriptome study comparing peri-implantitis with periodontitis included in this systematic review also showed that

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the MAPK1/3 signaling pathway was significantly enriched [99]. Members of the MAPK subfamilies, which are activated, for example, by stimulated pattern recognition receptors, play a role in innate immunity and the inflammatory response following tissue damage or pathogen infection [251]. Hence, the more intense immune response in peri-implantitis compared with periodontitis may be mediated by the MAPK signaling pathway and is under ceRNA control. Concordantly, in an animal study included in PICO 2b, target genes of differentially expressed miRNAs were linked to the MAPK cascade in experimental peri-implantitis compared to healthy tissue [94]. One potentially explanation of enhanced MAPK signaling pathway and associated immune response in peri-implantitis compared to its gingival counterpart could be the implant material itself. This is indicated by *in vitro* studies, with titanium particles altering gene expression in the MAPK signaling pathway and the subsequent chemokine response [79,83,133]. Furthermore, there is evidence in the literature that lncRNA and miRNA control wear particle-induced osteolysis around orthopedic implants [252,253]. Hence, these epigenetic modulators may also play a role in the pathogenesis of peri-implantitis.

Interestingly, MAPK pathways are also involved in other epigenetic processes such as the regulation of DNA methylation enzymes and histone modifications [254,255]. Because DNA methylation regulates bone resorption – as reflected by 3,515 differentially methylated genes in monocytes and osteoclasts during osteoclastogenesis [256] – this epigenetic mechanism could also play a role in inflammatory gum diseases with alveolar bone loss. As reviewed by Jiang et al., DNA methylation-induced changes in gene expression of cytokines/chemokines, toll-like receptors or extracellular matrix molecules disrupt the homeostasis of the periodontal tissue and its integrity as well as affect the immune response in periodontitis [257]. These modifications in DNA methylation can be triggered by periodontal pathogens but also by environmental factors such as smoking, diabetes or aging [257].

Thus, it is also conceivable that titanium wear of dental implants causes DNA methylation changes in their environment, which may promote peri-implantitis. Although mechanistic studies with oral-related cells provide little evidence of titanium-

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induced global DNA hypomethylation, a study examining tissue samples from patients with healthy and inflamed implants indicated that DNA hypermethylation is associated with higher titanium levels [235]. This correlation was still robust when adjusting for other environmental factors such as smoking and diabetes [235]. Only one study examining the DNA methylation pattern of peri-implantitis relative to periodontitis was suitable for this systematic review [97]. DNA methylation profiles created by reduced representation bisulfite sequencing differed slightly between the two diseases, with GO enrichment analyses of the differentially methylated genes yielding no significant results [97].

Different environmental conditions such as the implant material itself between peri-implantitis and periodontitis provide explanations for a distinct epigenetic regulation of the diseases and a few studies give indications for this. Nevertheless, further studies investigating diverse epigenetic mechanisms between peri-implantitis and periodontitis are needed, ideally with simultaneous measurement of the titanium content.

3.1.9.3 Genome Studies

In addition to multiple environmental influences, genetic variations among patients causing individual inflammatory responses may also be a factor in the pathogenesis of peri-implantitis [51,258]. For instance, certain individuals demonstrate increased sensitivity to titanium evidenced by elevated cytokine secretion of TNF- α and IL-1 β in whole blood primary cell cultures exposed to titanium dioxide; this heightened response was more pronounced in patients experiencing implant loss compared to individuals with healthy implants [51,259]. These findings were also apparent at genomic level, with a significant association found between implant failure and the combined polymorphisms in IL1A, IL1B, IL1RN, and TNFA [51,259]. Several other studies can be found in the literature examining single polymorphisms of genes associated with peri-implantitis that were previously selected based on plausible biological effects on this disease [260]; but generally with small sample sizes, resulting in low reproducibility and limited statistical power [260]. In contrast,

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genomic approaches are used to identify (novel) genetic variations of the entire genome that are associated with diseases [48]. However, based on the criteria defined in the PICO questions, no qualifying studies with genomic analyses were identified [51]. In a whole-exome sequencing pilot study of saliva samples from six patients with frequent implant failure caused by severe peri-implantitis, selected transcripts with genetic variation were linked to biological processes like cell adhesion, metal ion binding, and cytoskeleton playing a role in pathogenesis of peri-implantitis [51,261]. A microarray-based comparative genomic hybridization analysis of 30 and 20 blood samples from peri-implantitis patients and patients with healthy implants detected a heterozygote duplication of a genome portion matching exon 6 of the VEGFA gene in all peri-implantitis samples but not in the control group [220]. As important regulators of angiogenesis, vascular endothelial growth factors (VEGFs) maintain homeostasis and bone repair, among other functions [262]. Thus, the authors suggested that the participation of VEGF in bone regeneration during peri-implantitis is diminished due to a genetic deletion [51,220]. Nevertheless, further studies are needed to verify the role of VEGF in the pathogenesis of peri-implantitis and its genetic determinants, while there are conflicting results in the literature about the up- or downregulation of VEGF in association with peri-implantitis [262]. To achieve a valid conclusion regarding the genetic factors influencing peri-implantitis, also in contrast to periodontitis, further research is required analyzing the entire genome with a significantly larger sample and additional verification [51].

3.2 Comparison of a Large-Scale Transcriptome Study and Results of the Systematic Review

As discussed in section 3.1.1.4, the results of the studies included in the systematic review that examined transcriptome changes in peri-implantitis and periodontitis are potentially limited by their small sample size. Hence, they were cross-checked with the unpublished results of a large-scale study that investigated the transcriptome of 90 untreated peri-implantitis and periodontitis sites. Furthermore, these results were compared to the functional enrichment profile and DEG of cells exposed to titanium

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particles to verify a potential overlap with altered biological processes and gene expression in peri-implantitis. In the following, possible reasons for different results in functional enrichment and gene expression analyses of individuals with peri-implantitis are presented. In addition to the potential negative effects of titanium on peri-implantitis, recent improvements in implant design will be discussed.

3.2.1 Comparison with Transcriptome Studies Examining the Differences between Peri-Implantitis and Peri-odontitis

Since a list of all DEG could only be obtained from one of four studies, the results of all studies were compared based on the reported functional enrichment results. Generally, tools for functional enrichment analysis can be classified into two categories: over-representing analysis (ORA) and functional class scoring (FCS) [263]. ORA analyses are widely used because of its simplicity of implementation as it is based on well-established statistical methods with DEG lists as inputs [264]. Using Fisher's exact test or Hypergeometric test, it is tested whether the input DEG are overrepresented in a pathway or gene set compared to a randomly generated distribution of genes from a background list [263]. In contrast, FCS is performed on gene expression data without a priori selection of differentially expressed genes. Thus, it is more difficult to apply, but more sensitive in detection of subtle associations [263]. Gene set enrichment analysis (GSEA) is one widely used approach which measures the association between members of a gene set and the test group of the gene expression experiment (enrichment score) using all previously ranked genes [264]. These methods – ORA and FCS – achieved a moderated concordance while performing both functional enrichment analysis methods on seven independent RNA-Seq datasets [263].

Although the large-scale study and the results of the studies with small sample sizes exhibited a common functional profile at a fairly global level with genes enriched in processes such as immune response, metabolism and cell adhesion, the overlap of more specific GO terms and pathways was considerably less. However,

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pathways that have been recurrently identified in multiple studies are likely to be more reliable than those derived from a single analysis of a single data set [124]. For instance, pathways such as ‘Extracellular matrix organization’, ‘ECM-receptor interaction’ or ‘Cytokine-cytokine receptor interaction’ might be important in the development and progression of peri-implantitis as extracellular matrix (ECM) adhesion proteins and cytokines play a critical role in modulating the inflammatory reaction to implant materials [265]. Consistently, processes like ‘Cell adhesion and cell junction organization’ and ‘Cell-matrix adhesion’ were top ranked in the functional annotation clustering analysis comparing peri-implantitis and periodontitis based on the data of the large-scale study.

Nevertheless, this comparison is limited due to the selective reporting of enriched GO terms and pathways. For example, Zhou et al. reported only the top ten GO terms related to the DEG from RNA sequencing analysis [99]. Moreover, different methods for functional enrichment analysis were used, with their quality highly depending on the quality of input (i.e., DEG lists) [266]. Thus, the choice of the reference genome to which the mapped DEG of a gene set are compared can heavily influence the enrichment results; for instance, an overly large background set can lead to more significantly altered biological processes than actually affected [266,267]. A recent review of 186 research articles highlighted many incorrect applications of functional enrichment analysis [263]. These include inappropriate background gene lists or no multiple testing corrections, and a lack of detailed methodology description leading to unreliable results [263]. This also applied to almost all studies that were eligible for PICO question 3c. Therefore, a guideline such as the MIAME guideline that exists for reporting microarray data [120] is highly recommended to improve reproducibility [263].

A comparison of results obtained by the same gene set enrichment analysis with data from the MAQC project indicated that differences in microarray platforms and selection criteria for the given DEG list did not affect the concordance of GO terms [268]. Based on a rat toxicogenomics data set generated by four distinct microarray platforms, Guo et al. demonstrated that the overlap of GO terms as well

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as pathways across platforms is high when using a DEG list selected by fold changes and p-values [269]. Hence, the algorithm provided in the `clusterProfiler` package was applied to DEG lists (with fold change and p-value cutoff) of different studies to compare their GO and pathway profiles using the same enrichment analysis method. Therefore, the over-representing analysis based on a Fisher's exact test was applied since Manoli et al. showed that this method – in comparison to GSEA – yielded the most consistent results obtained from gene lists generated by different statistical methods and from data sets of three studies performed with different microarray platforms [124]. However, the functional profile of the large-scale transcriptome study examining the difference of peri-implantitis and periodontitis could only be compared with one other study [75] via the `clusterProfiler` algorithm. Thereby, no overlap was apparent.

Because the difference in methodology in these two studies tends to play a minor role, the considerably smaller sample size in the study included in the systematic review offers potential explanation for the difference in results as this produces more unreliable results. Furthermore, the complexity of the multifactorial disease peri-implantitis and the heterogeneity of host responses can lead to inter-study discrepancy. This is also highlighted by a comparative analysis of two cohort studies testing the transcriptome of the similar gum disease periodontitis by calculating the overlap of separately created DEG lists and their enriched GO terms [270]. The authors conclude that the reliability of the studies was quite low both at the level of DEG and biological processes [270]. The DEG list of two other studies also overlapped only slightly with the respective cohort studies, ranging from 0.4 (1 gene) to 26% [270]. This is consistent with the observed small overlap of DEG (6.8%) in the two studies investigating the transcriptomic difference between peri-implantitis and periodontitis. Another reason for the varying study results could be the different handling of confounding factors. While Liu et al. only calculated the between-group differences (peri-implantitis vs periodontitis) regarding age and probing depth, the large-scale study controlled the confounding variable 'probing depth' by matching. Additionally, the confounder 'sex' was included in the regression model used for

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the differential expressed gene analysis. Besides technical variations (see section 3.1.1), gene expression estimates are influenced by biological factors such as sex, age or ancestry as well as the cell composition of the samples [271–274]. After identifying important confounding variables, these should be controlled either by study design (e.g., matching, randomization or restriction) or statistical analysis (e.g., stratification or multivariate analysis) [275]. For instance, a split-mouth design with sampling of peri-implant and periodontal tissue at a similar site in the same patient is beneficial.

Consequently, more studies are needed especially with a sufficiently large sample size to draw more reliable conclusions about the differences in gene expression in peri-implantitis and periodontitis. Moreover, a uniform study design, for example, in terms of diagnostic criteria of gingival diseases and an adequate control of confounding factors is required. In addition, a provision of raw gene expression data is desirable to facilitate the integration of multiple studies and thereby increase the reliability and power of the results.

3.2.2 Comparison with Transcriptome Studies Examining the Impact of Titanium Particles on Cells

Despite a similarly low proportion of overlapping DEG, the `clusterProfiler` analysis found an, albeit modest, overlap in the functional profile of peri-implantitis in comparison to periodontitis and in cells subjected to titanium particles. In particular, common pathways linked to inflammatory host response mediated by cytokines and GO terms related to cytokine/chemokine response were enriched in peri-implantitis relative to periodontitis affected tissue and titanium exposed cells. This supports the hypothesis of the preceding systematic review that the observed different molecular patterns between peri-implantitis and periodontitis are also linked to titanium wear debris. “A potential mechanism is the amplification of the adverse effects of an exuberant immune response” [51], which is reflected histopathologically in a higher proportion of neutrophils in peri-implantitis [22]. As indicated at the functional enrichment level, chemokines such as CXCL2, CCL3

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and CCL7 were both upregulated in peri-implantitis and oral related cells treated with titanium particles. Moreover, CXCL2 was detected in other animal and human studies investigating the transcriptome of tissue around inflamed or healthy implants [92,220,221]. Since CXCL2 levels were not affected or decreased by adding a monospecies (*S. oralis* or *A.a.*) or multispecies biofilm to an *in vitro* peri-implant mucosa model [276,277], the observed enhanced levels of chemokines in peri-implantitis in comparison to periodontitis could be explained by another factor such as a foreign body reaction to released titanium wear debris from implants. Additionally, the stronger participation in detoxification of metal particles of cells in tissues surrounding inflamed implants than inflamed teeth is reflected by the upregulation of family members of metallothionein (MT2A, MT1G, MT1H). These were also similarly expressed in multiple studies examining titanium or other metallic exposure to cell lines related to the oral cavity (see section 3.1.5).

In order to minimize the adverse immune response as well as metal wear debris, ongoing enhancements in implant design are beneficial, e.g., optimization of the surface chemical properties or surface coating [17,265]. These techniques are advantageous as they can improve biocompatibility and preserve the bulk properties of the implant [278]. Current investigations into implantable biomaterials show a shift from bioinert implants to bioactive designs, aiming to elicit a favorable immune response [17,265,279]. In the field of dentistry, few concepts exist for stimulating bone regeneration and avoiding the formation of peri-implantitis [17,280–282]. An intriguing example is provided by the immobilization of the antimicrobial peptide GL13K onto a silanized titanium implant [283]. This showed immunomodulatory properties by downregulating pro-inflammatory and upregulating anti-inflammatory cytokines and inhibiting the activation of macrophages as well as promoting the M2 macrophage polarization [283]. Recent research has demonstrated that GL13K coating stimulates osteogenesis, angiogenesis, and antiosteoclastogenesis via osteoimmunomodulation [284]. Furthermore, this surface modification reached a strong corrosion resistance which is favorable with regard to titanium release into the surrounding tissue [284]. Since the host immune response varies greatly from

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individual to individual and depends, for example, on age [265], personalized implant therapy gains more importance in research [278]. For instance, patient derived monocytes can be exposed to different implant surfaces (e.g., coating/uncoating) to evaluate the individual macrophage response *ex vivo* and subsequently choose the appropriate biomaterial based on the patient response [278]. The high biocompatibility of implants made of zirconium dioxide, evidenced by reduced bacterial colonization, weaker inflammatory reaction, and greater level of angiogenic and anti-inflammatory cytokines in comparison to titanium implants, leads to increasing discussions about the potential of zirconia implants serving as an alternative to titanium implants [17,285]. While this indicates a lower incidence of peri-implantitis associated with zirconia implants, there is a lack of comparative long-term studies involving zirconia as well as titanium implants [17,285].

3.3 Limitations

As none of the studies incorporated in the systematic review investigated both the titanium content level and the discrimination of the transcriptome in peri-implantitis and periodontitis simultaneously, any correlation can only be assumed, given that titanium particles induced similar alterations in biological processes in *in vitro* experiments [51]. Nonetheless, several studies reported increased titanium concentrations in tissues around inflamed implants, in contrast to both healthy implants and teeth affected by periodontitis [30,32–36,51].

Comparing *in vitro* and *in vivo* studies is limited because cellular investigations cannot exactly model the entire tissue processes as the complexity and interaction of various cells cannot be imitated [51,100]. Nevertheless, cell lines, such as primary fibroblasts, display a gene expression signature similar to the tissue from which they originate (like skin in this case), albeit at a lower level [51,100].

Furthermore, titanium nanoparticles were mainly used in cellular studies, which may not represent conditions in peri-implant tissues. For instance, Pettersson et al. identified titanium with particle mean sizes corresponding to macro- and

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microparticles as well as submicron particles – but no nanoparticles – via transmission electron microscopy in peri-implantitis samples [194]. Consistently, titanium particles between 0.5 and 40 μm were found in jawbone marrow tissues around dental implants [33]. However, the detection limit of the used methodology was 0.5 μm and, therefore, the presence of nanoparticles cannot be excluded [33]. In principle, nanoparticles are more biologically active and thus potentially more harmful than microparticles due to their larger surface-to-volume ratio [286]. However, since nanoparticles have the ability to aggregate to sizes like microparticles, they can also exhibit similar behavior [286]. For example, an *in vitro* study investigating the transcriptome of colon cells exposed to six titanium particles with different sizes showed that nanoparticles aggregated to hydrodynamic sizes above 100 nm in culture medium. Herein, agglomerated particle size correlated better with transcriptomic response than dry particle size [102].

Despite this, the cells of the selected studies were exposed to titanium concentrations (mean: 69 $\mu\text{g}/\text{ml}$, range: 0.5 to 300 $\mu\text{g}/\text{ml}$) that were similar to the measured titanium levels in peri-implantitis tissue (mean: 98.7 $\mu\text{g}/\text{g}$, range: 10.6 to 340.3 $\mu\text{g}/\text{g}$) [194]. However, the majority of the identified studies focused on the impact of titanium on cells for a brief period of time, failing to adequately capture the effects of prolonged implant wear on the adjacent tissue [17]. Therefore, studies with long-term exposure are required to verify the reported cellular changes in oral tissue [17]. Two studies investigating the proteome of lung cells for 13 weeks or two months [134,138], offer an indication in this regard [17]. These studies indicated an essential role of stress responses and cell death pathways in long-term cellular reactions to titanium particles and therefore impacting cellular functions similarly to those observed in acute exposure [17,134,138]. A similar conclusion was reached by the authors of an epigenome study with a lung epithelial cell line exposed to titanium for two and four weeks, where exposure time had no effect and only a slight change in genome-wide DNA methylation by titanium was detected [160].

It is also noteworthy that certain cell studies were unable to detect changes in gene expression caused by titanium [51,80,85,86]. Possible explanations were

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discussed in section 3.1.1 including a variety of study designs, diverse statistical analysis with different cutoff criteria and technical variations ('batch-effects') [17,51]. Nevertheless, further omics studies, especially with adequate sample size, are required to verify the cellular effects of titanium.

Since a systematic review's quality relies on the quality of its integrated studies, the statements provided should be interpreted cautiously [17]. On the one hand, the small sample size without a priori power analyses generally reduces the strength of the studies. On the other hand, the insufficient description of the statistical analyses, especially in the animal studies, increases the risk of bias resulting in less reliable results. No controlling of confounders in sense of matching or incorporating in statistical analysis also decreases the reliability of the results in human studies. Furthermore, raw data or as an minimum lists with all differentially expressed genes/proteins were only accessible in a limited number of studies complicating the integration of the results from diverse reports leading to less powerful outputs (see section 3.1.2) [51]. This also applies to the functional enrichment analyses, and hence, only broad conclusions can be made by comparing partially selectively reported results of the GO and pathway enrichment analyses (see section 3.2.1).

In addition, it should be noted that the observed changes in gene expression do not necessarily occur at the protein level, which can be assumed by the low genome-wide correlation between mRNAs and their protein products, for example, via post-transcriptional modifications [287]. However, when considering differentially expressed genes compared to non-differentially expressed genes, DEG have a significantly higher correlation to their protein products [287]. This supports the assumptions that results of DEG analysis reflect also differences at the functional level of proteins [287].

Nonetheless, as 'multi-omics' approaches integrate different omics data types, they are useful for understanding complex and causal biological processes from a holistic perspective [48,288]. For example, Overmyer and colleagues analyzed proteomics, lipidomics, metabolomics and 16S DNA sequencing data of dental plaque

samples from 97 healthy, periodontitis or diabetes patients [289]. Cross-omic correlation analysis revealed an association between proteins and specific metabolite/lipid profiles, whereas host-specific proteins and lipids such as phosphatidylcholines were highly correlated in plaque samples of periodontitis-affected patients [289]. Moreover, this multi-omics approach identified a novel lipid pathway in the oral bacterium *Lautropia mirabilis* and a diet-related association between proteins and lipids in dental plaque highlighting the discovery potential of integrative omics analysis [289].

Conclusion

Since peri-implantitis is a multifactorial disease and varies between patients due to host-specific immune responses [258], causal relationships with regard to the pathogenesis of peri-implantitis are difficult to identify. As peri-implantitis is clinically and histopathologically distinct from periodontitis, the implant material itself was suspected as a possible factor in the development and progression of this disease [4]. Although omics technologies provide a holistic view to complex biological processes, single analyses of one omics data type can only prove a correlation but not a causality [48]. Therefore, the integration of multiple omics approaches is preferable in future research.

A significant overlap of differentially expressed genes across various titanium-exposure cell studies was evident using Monte Carlo simulation and subsequently verified by robust rank aggregation [17]. However, the resulting ranking of commonly expressed genes should be interpreted with caution as the analyses were based on previously compiled DEG lists rather than integrated raw gene expression data.

Despite the limited number of quite heterogeneous studies, common transcriptome and proteome changes across diverse cell types exposed to titanium particles support the hypothesis that the observed different molecular patterns between peri-implantitis and periodontitis are also linked to titanium wear debris [17]. Amplifying the harmful impacts of an excessive immune response stimulated by both the toll-like receptor signaling pathway and MAPK signaling pathway could be one potential mechanism [51]. Furthermore, the functional enrichment profile of a large-scale transcriptome study comparing peri-implantitis and periodontitis substantiates the assumption of an enhanced inflammatory host response. A potential mediator are cytokines possibly induced by the interaction of ECM adhesion proteins with the

implant material. Consequently, the effect of an immunomodulating coating of titanium implants or implants made of zirconia on the onset of peri-implantitis is worth studying.

Because of the limited amount of studies analyzing just a subset of possible epigenetic changes and their contradictory outcomes, it is challenging to conclude definitively on the epigenetic impact of titanium on cells and if this affects the differences between peri-implantitis and periodontitis [17].

To obtain reliable and reproducible results, researchers ought to focus on choosing an appropriate sample size through a priori power analyses [17]. In the context of transcriptome studies, Orr et al. and Zhao et al. provide a useful starting point [113,114]. In addition, statistical methods for differentially expressed genes/proteins and functional enrichment analyses should be described in detail, and re-analyzable gene/protein expression data should be supplied [17]. Adequate control of confounding factors such as sex, age or probing depth in *in vivo* studies is also beneficial.

Additional omics studies, which concurrently analyze titanium content across various levels (like transcriptome, proteome, epigenome, genome, metabolome), are necessary to verify the impact of titanium implant wear debris on molecular alterations in peri-implant tissues [17].

Appendices



Reasons for Exclusion

Reasons for exclusion of the 23 articles after full text screening in the systematic review:

| Study | DOI | Reason |
|----------------|------------------------------|--|
| Becker, 2014 | 10.1111/cid.12001 | does not match the PICO conditions (one smoker included) |
| Bressan, 2019 | 10.3390/ma12122036 | sampling not at least one year after implantation (in control group) |
| Brzicova, 2017 | 10.1016/j.toxlet.2017.08.044 | only abstract available |
| Carinci, 2003 | 10.1002/jbm.b.10021 | no Ti particles used |
| Chen, 2018 | 10.1016/j.redox.2017.12.011 | no human cell line |
| Daubert, 2019 | 10.1177/2380084418822831 | does not match the PICO conditions (smokers and systematic disease) |
| Ishikawa, 2021 | 10.2334/josnurd.21-0130 | no human genome as reference genome in SAGE analysis |
| Lee, 2014 | 10.1371/journal.pone.0099360 | pilot study; does not match the group of PICO (control group not well defined) |
| Lewallen, 2017 | 10.1002/jbmr.3107 | only abstract available |
| Li, 2020 | 10.1186/s12881-020-01145-4 | does not match the groups of PICOs |
| Nahm, 2015 | 10.1155/2015/538080 | does not match the groups of PICOs |
| Omidi, 2020 | 10.1039/d0mt00028k | no Ti particles used |
| Palmieri, 2007 | 10.1016/j.nano.2007.03.004 | not no titanium as control |

A. Reasons for Exclusion

| Study | DOI | Reason |
|-------------------|-------------------------------|--|
| Palmieri, 2008 | 10.1097/SCS.0b013e3181534ab3 | no Ti particles used |
| Peng, 2010 | 10.1021/nl903043z | does not match the groups of PICOs |
| Poon, 2020 | 10.1080/17435390.2019.1687776 | wrong outcome |
| Schminke, 2015 | 10.1177/0022034514559128 | does not match the PICO conditions (smokers and systematic disease) |
| Sollazzo, 2008 | 10.1002/jbm.b.30912 | not no titanium as control |
| Urvasizoglu, 2021 | 10.11607/jomi.8470 | does not match the PICO conditions (smokers and < 1 year follow-up) |
| Vračko, 2018 | 10.1080/1062936X.2018.1498015 | wrong method (similarity index); same data as Tilton et al. (2014) |
| Yang, 2021 | 10.1016/j.jot.2020.10.004 | no oral implant |
| Yim, 2012 | 10.1007/s13273-012-0028-2 | not a in vitro study (in silico, cross-sectional) + no cell line corresponding to a peri-implantitis model |
| Zhang, 2017 | 10.3892/etm.2017.4176 | does not match the PICO conditions (smokers and systematic disease) |

B

Full Search Strategy

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|---|----------------------|
| 1a | <p>(("cells, cultured"[MeSH Terms] OR "cells cultured"[Title/Abstract] OR "endothelial cells"[Title/Abstract] OR "epithelial cells"[Title/Abstract] OR "tissue cells"[Title/Abstract] OR "tissue culture"[Title/Abstract] OR "vitro studies"[Title/Abstract] OR "vitro study"[Title/Abstract] OR "human cell culture"[Title/Abstract] OR "human cell*"[Title/Abstract] OR "gingiva*"[Title/Abstract] OR "fibroblast*"[Title/Abstract] OR "odontoblast*"[Title/Abstract] OR "osteoclast*"[Title/Abstract] OR "osteoblast*"[Title/Abstract] OR "human monocytes"[Title/Abstract] OR "macrophage*"[Title/Abstract]) AND ("titanium alloy"[Title/Abstract] OR "titanium alloys"[Title/Abstract] OR "titanium dental"[Title/Abstract] OR "titanium dioxide"[Title/Abstract] OR "titanium discs"[Title/Abstract] OR "titanium particle*"[Title/Abstract] OR "titanium substrate"[Title/Abstract] OR "titanium nanoparticle*"[Title/Abstract] OR "implant particle*"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microRNA*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ("english"[Language] OR "german"[Language])</p> | 1,043 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 1b | (("cells, cultured"[MeSH Terms] OR "cells cultured"[Title/Abstract] OR "endothelial cells"[Title/Abstract] OR "epithelial cells"[Title/Abstract] OR "tissue cells"[Title/Abstract] OR "tissue culture"[Title/Abstract] OR "vitro studies"[Title/Abstract] OR "vitro study"[Title/Abstract] OR "human cell culture"[Title/Abstract] OR "human cell*"[Title/Abstract] OR "gingiva*"[Title/Abstract] OR "fibroblast*"[Title/Abstract] OR "odontoblast*"[Title/Abstract] OR "osteoclast*"[Title/Abstract] OR "osteoblast*"[Title/Abstract] OR "human monocytes"[Title/Abstract] OR "macrophage*"[Title/Abstract]) AND ("titanium ion"[Title/Abstract] OR "titanium ions"[Title/Abstract] OR "ti ion"[Title/Abstract] OR "ti ions"[Title/Abstract] OR "ppm ti"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microRNA*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ("english"[Language] OR "german"[Language]) | 27 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 1c | <p>((("cells, cultured"[MeSH Terms] OR "cells cultured"[Title/Abstract] OR "endothelial cells"[Title/Abstract] OR "epithelial cells"[Title/Abstract] OR "tissue cells"[Title/Abstract] OR "tissue culture"[Title/Abstract] OR "vitro studies"[Title/Abstract] OR "vitro study"[Title/Abstract] OR "human cell culture"[Title/Abstract] OR "human cell*"[Title/Abstract] OR "gingiva*"[Title/Abstract] OR "fibroblast*"[Title/Abstract] OR "odontoblast*"[Title/Abstract] OR "osteoclast*"[Title/Abstract] OR "osteoblast*"[Title/Abstract] OR "human monocytes"[Title/Abstract] OR "macrophage*"[Title/Abstract]) AND ("corrosion product*"[Title/Abstract] OR "metal oxide"[Title/Abstract] OR "metal particle*"[Title/Abstract] OR "metal ion"[Title/Abstract] OR "metal ions"[Title/Abstract] OR "metal nanoparticles"[MeSH Terms] OR "cobalt"[MeSH Terms] OR "chromium"[MeSH Terms] OR "aluminum"[MeSH Terms] OR "nickel"[MeSH Terms] OR "vanadium"[MeSH Terms] OR "magnesium"[MeSH Terms] OR "implant particle*"[Title/Abstract] OR "cobalt"[Title/Abstract] OR "chromium"[Title/Abstract] OR "aluminium"[Title/Abstract] OR "nickel"[Title/Abstract] OR "vanadium"[Title/Abstract] OR "magnesium"[Title/Abstract]) AND ("titanium"[MeSH Terms] OR "titanium*"[Title/Abstract] OR "Ti"[Title/Abstract] OR "ti3"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ("english"[Language] OR "german"[Language]))</p> | 488 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|---|----------------------|
| 1d | <p>(("cells, cultured"[MeSH Terms] OR "cells cultured"[Title/Abstract] OR "endothelial cells"[Title/Abstract] OR "epithelial cells"[Title/Abstract] OR "tissue cells"[Title/Abstract] OR "tissue culture"[Title/Abstract] OR "vitro studies"[Title/Abstract] OR "vitro study"[Title/Abstract] OR "human cell culture"[Title/Abstract] OR "human cell*"[Title/Abstract] OR "gingiva*"[Title/Abstract] OR "fibroblast*"[Title/Abstract] OR "odontoblast*"[Title/Abstract] OR "osteoclast*"[Title/Abstract] OR "osteoblast*"[Title/Abstract] OR "human monocytes"[Title/Abstract] OR "macrophage*"[Title/Abstract]) AND ("actinomyces naeslundii"[Title/Abstract] OR "aggregatibacter actinomycetemcomitans"[MeSH Terms] OR "campylobacter rectus"[MeSH Terms] OR "fusobacterium nucleatum"[MeSH Terms] OR "porphyromonas gingivalis"[MeSH Terms] OR "prevotella intermedia"[MeSH Terms] OR "tannerella forsythia"[MeSH Terms] OR "treponema denticola"[MeSH Terms] OR "periodontal pathogens"[Title/Abstract] OR "lipopolysaccharide"[Title/Abstract] OR "lipopolysaccharides"[MeSH Terms] OR "LPS"[Title/Abstract] OR "oral bacteria"[Title/Abstract] OR "p gingivalis"[Title/Abstract]) AND ("titanium"[MeSH Terms] OR "titanium*"[Title/Abstract] OR "Ti"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND (english[Filter] OR german[Filter])</p> | 150 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 2a | <p>((("animal experimentation"[MeSH Terms] OR "animal experiment*" [Title/Abstract] OR "vivo study"[Title/Abstract] OR "vivo studies"[Title/Abstract] OR "animal"[Title/Abstract] OR "rat"[Title/Abstract] OR "rats"[MeSH Terms] OR "mouse"[Title/Abstract] OR "mice"[MeSH Terms] OR "dog"[Title/Abstract] OR "dogs"[MeSH Terms] OR "rabbit"[Title/Abstract] OR "rabbits"[MeSH Terms] OR "rats"[Title/Abstract] OR "mice"[Title/Abstract] OR "dogs"[Title/Abstract] OR "rabbits"[Title/Abstract]) AND ("implant*" [Title/Abstract] OR "peri-implant"[Title/Abstract]) AND ("tooth"[Title/Abstract] OR "tooth"[MeSH Terms] OR "teeth"[Title/Abstract] OR "healthy teeth"[Title/Abstract] OR "oral mucosal tissue*" [Title/Abstract] OR "junctional epithelium"[Title/Abstract] OR "periodontal connective tissue*" [Title/Abstract] OR "periodontal tissue*" [Title/Abstract] OR "healthy tissue*" [Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*" [Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*" [Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*" [Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*" [Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*" [Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*" [Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*" [Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*" [Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*" [Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND (english[Filter] OR german[Filter])</p> | 263 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 2b | <p>((("animal experimentation"[MeSH Terms] OR "animal experiment*" [Title/Abstract] OR "vivo study"[Title/Abstract] OR "vivo studies"[Title/Abstract] OR "animal"[Title/Abstract] OR "rat"[Title/Abstract] OR "rats"[MeSH Terms] OR "mouse"[Title/Abstract] OR "mice"[MeSH Terms] OR "dog"[Title/Abstract] OR "dogs"[MeSH Terms] OR "rabbit"[Title/Abstract] OR "rabbits"[MeSH Terms] OR "rats"[Title/Abstract] OR "mice"[Title/Abstract] OR "dogs"[Title/Abstract] OR "rabbits"[Title/Abstract]) AND ("peri implantitis"[MeSH Terms] OR "peri-implantitis"[Title/Abstract] OR "periimplantitis"[Title/Abstract] OR "peri implant disease*" [Title/Abstract] OR "peri implant osteolysis"[Title/Abstract] OR "failing dental implant*" [Title/Abstract]) AND ("peri-implant"[Title/Abstract] OR "implant*" [Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*" [Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*" [Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*" [Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*" [Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*" [Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*" [Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*" [Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*" [Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*" [Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND (english[Filter] OR german[Filter]))</p> | 92 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 2c | <p>((("animal experimentation"[MeSH Terms] OR "animal experiment*"[Title/Abstract] OR "vivo study"[Title/Abstract] OR "vivo studies"[Title/Abstract] OR "animal"[Title/Abstract] OR "rat"[Title/Abstract] OR "rats"[MeSH Terms] OR "mouse"[Title/Abstract] OR "mice"[MeSH Terms] OR "dog"[Title/Abstract] OR "dogs"[MeSH Terms] OR "rabbit"[Title/Abstract] OR "rabbits"[MeSH Terms] OR "rats"[Title/Abstract] OR "mice"[Title/Abstract] OR "dogs"[Title/Abstract] OR "rabbits"[Title/Abstract]) AND ("peri implantitis"[MeSH Terms] OR "peri-implantitis"[Title/Abstract] OR "periimplantitis"[Title/Abstract] OR "peri implant disease*"[Title/Abstract] OR "peri implant osteolysis"[Title/Abstract] OR "failing dental implant*"[Title/Abstract]) AND ("periodontal diseases"[MeSH Terms] OR "periodontal disease*"[Title/Abstract] OR "periodontitis"[MeSH Terms] OR "periodontitis"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND (english[Filter] OR german[Filter])</p> | 40 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 3a | <p>((("healthy patients"[Title/Abstract] OR "human*"[Title/Abstract] OR "humans"[MeSH Terms] OR "patients"[MeSH Terms] OR "individuals"[Title/Abstract]) AND ("implant*"[Title/Abstract] OR "peri-implant"[Title/Abstract]) AND ("healthy teeth"[Title/Abstract] OR "healthy tissue*"[Title/Abstract] OR "natural teeth"[Title/Abstract] OR "periodontal tissue*"[Title/Abstract] OR "teeth"[Title/Abstract] OR "tooth"[MeSH Terms] OR "tooth"[Title/Abstract] OR "junctional epithelium"[Title/Abstract] OR "periodontal connective tissue*"[Title/Abstract] OR "oral mucosal tissue*"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ((english[Filter] OR german[Filter]))</p> | 286 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|--|----------------------|
| 3b | (("healthy patients"[Title/Abstract] OR "human*"[Title/Abstract] OR "humans"[MeSH Terms] OR "patients"[MeSH Terms] OR "individuals"[Title/Abstract]) AND ("peri implantitis"[MeSH Terms] OR "periimplantitis"[Title/Abstract] OR "peri-implantitis"[Title/Abstract] OR "peri implant disease*"[Title/Abstract] OR "peri implant osteolysis"[Title/Abstract] OR "failing dental implant*"[Title/Abstract]) AND ("implant*"[Title/Abstract] OR "peri-implant"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microRNA*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ((english[Filter] OR german[Filter])) | 262 |

^aNumber of articles found after the first search on 07/01/2021

| PECO | Search string for MEDLINE (through Pubmed) builded in Pubmed | Results ^a |
|------|---|----------------------|
| 3c | <p>((("healthy patients"[Title/Abstract] OR "human*"[Title/Abstract] OR "humans"[MeSH Terms] OR "patients"[MeSH Terms] OR "individuals"[Title/Abstract]) AND ("peri implantitis"[MeSH Terms] OR "periimplantitis"[Title/Abstract] OR "peri-implantitis"[Title/Abstract] OR "peri implant disease*"[Title/Abstract] OR "peri implant osteolysis"[Title/Abstract] OR "failing dental implant*"[Title/Abstract])) AND ("periodontal diseases"[MeSH Terms] OR "periodontal disease*"[Title/Abstract] OR "periodontitis"[MeSH Terms] OR "periodontitis"[Title/Abstract]) AND ("cellular response"[Title/Abstract] OR "inflammatory response"[Title/Abstract] OR "protein level"[Title/Abstract] OR "gene expression"[MeSH Terms] OR "gene expression profiling"[MeSH Terms] OR "transcriptome"[MeSH Terms] OR "transcriptome"[Title/Abstract] OR "transcriptomic*"[Title/Abstract] OR "rna seq"[MeSH Terms] OR "rna seq"[Title/Abstract] OR "genome wide"[Title/Abstract] OR "epigenomics"[MeSH Terms] OR "epigenomic*"[Title/Abstract] OR "epigenetics"[Title/Abstract] OR "expressed genes"[Title/Abstract] OR "microarray analysis"[MeSH Terms] OR "microarray*"[Title/Abstract] OR "genes expressed"[Title/Abstract] OR "mrna*"[Title/Abstract] OR "RNA"[Title/Abstract] OR "RNAs"[Title/Abstract] OR "microrna*"[Title/Abstract] OR "dna damage"[MeSH Terms] OR "dna damage"[Title/Abstract] OR "methylation"[MeSH Terms] OR "methylation"[Title/Abstract] OR "histone acetylation"[Title/Abstract] OR "epigenetic changes"[Title/Abstract] OR "molecular mechanism"[Title/Abstract] OR "molecular signatures"[Title/Abstract] OR "proteome"[MeSH Terms] OR "proteome"[Title/Abstract] OR "proteomics"[MeSH Terms] OR "proteomic*"[Title/Abstract] OR "regulated proteins"[Title/Abstract] OR "protein production"[Title/Abstract] OR "expression*"[Title/Abstract] OR "high throughput"[Title/Abstract] OR "genome"[MeSH Terms] OR "genome"[Title/Abstract] OR "genomics"[MeSH Terms] OR "genomic*"[Title/Abstract] OR "polymorphism, genetic"[Mesh Terms] OR "polymorphism*"[Title/Abstract] OR "omic"[Title/Abstract] OR "omics"[Title/Abstract])) AND ((english[Filter] OR german[Filter]))</p> | 215 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 1a | <p>(‘human cell culture’/exp OR ‘human cell culture’ OR ‘epithelium cell’/exp OR ‘epithelium cell’ OR ‘endothelium cell’/exp OR ‘endothelium cell’ OR ‘tissue culture’/exp OR ‘tissue culture’ OR ‘human tissue, cells or cell components’/exp OR ‘human tissue, cells or cell components’ OR ‘gingiva’/exp OR ‘gingiva*’ OR ‘fibroblast’/exp OR ‘fibroblast*’ OR ‘odontoblast’/exp OR ‘odontoblast’ OR ‘osteoclast’/exp OR ‘osteoclast’ OR ‘osteoblast’/exp OR ‘osteoblast’ OR ‘macrophage’/exp OR ‘macrophage’ OR ‘human monocytes’:ab,ti OR ‘cells cultured’:ab,ti OR ‘vitro study’:ab,ti OR ‘vitro studies’:ab,ti OR ‘human cell*’:ab,ti) AND (‘titanium alloy’:ab,ti OR ‘titanium alloys’:ab,ti OR ‘titanium dental’:ab,ti OR ‘titanium dioxide nanoparticle’/exp OR ‘titanium dioxide nanoparticle’ OR ‘titanium discs’:ab,ti OR ‘titanium particle*’:ab,ti OR ‘titanium substrate’:ab,ti OR ‘titanium nanoparticle*’:ab,ti OR ‘titanium nanoparticle’/exp OR ‘titanium nanoparticle’ OR ‘implant particle*’:ab,ti) AND (‘cellular response’:ab,ti OR ‘inflammatory response’:ab,ti OR ‘protein level’:ab,ti OR ‘gene expression’/exp OR ‘gene expression’ OR ‘transcriptome’/exp OR ‘transcriptome’ OR ‘transcriptomics’/exp OR ‘transcriptomics’ OR ‘transcriptomic*’:ab,ti OR ‘rna sequencing’/exp OR ‘rna sequencing’ OR ‘rna seq’:ab,ti OR ‘genome wide’:ab,ti OR ‘epigenetics’/exp OR ‘epigenetics’ OR ‘epigenomic*’:ab,ti OR ‘expressed genes’:ab,ti OR ‘microarray analysis’/exp OR ‘microarray analysis’ OR ‘microarray*’:ab,ti OR ‘genes expressed’:ab,ti OR ‘messenger rna’/exp OR ‘messenger rna’ OR ‘mrna*’ OR ‘rna’/exp OR ‘rna’ OR ‘rnas’:ab,ti OR ‘microrna’/exp OR ‘microrna’ OR ‘dna damage’/exp OR ‘dna damage’ OR ‘methylation’/exp OR ‘methylation’ OR ‘epigenetic changes’:ab,ti OR ‘molecular mechanism’/exp OR ‘molecular mechanism’ OR ‘molecular signatures’:ab,ti OR ‘proteome’/exp OR ‘proteome’ OR ‘proteomics’/exp OR ‘proteomics’ OR ‘proteomic*’:ab,ti OR ‘protein production’/exp OR ‘protein production’ OR ‘expression*’:ab,ti OR ‘high throughput sequencing’/exp OR ‘high throughput sequencing’ OR ‘high throughput’:ab,ti OR ‘genome’/exp OR ‘genome’ OR ‘genomics’/exp OR ‘genomics’ OR ‘genomic*’:ab,ti OR ‘polymorphism*’:ab,ti OR ‘histone acetylation’/exp OR ‘histone acetylation’ OR ‘omics’/exp OR ‘omics’) AND ([english]/lim OR [german]/lim)</p> | 887 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 1b | ('human cell culture'/exp OR 'human cell culture' OR 'epithelium cell'/exp OR 'epithelium cell' OR 'endothelium cell'/exp OR 'endothelium cell' OR 'tissue culture'/exp OR 'tissue culture' OR 'human tissue, cells or cell components'/exp OR 'human tissue, cells or cell components' OR 'gingiva'/exp OR 'gingiva*' OR 'fibroblast'/exp OR 'fibroblast*' OR 'odontoblast'/exp OR 'odontoblast' OR 'osteoclast'/exp OR 'osteoclast' OR 'osteoblast'/exp OR 'osteoblast' OR 'macrophage'/exp OR 'macrophage' OR 'human monocytes':ab,ti OR 'cells cultured':ab,ti OR 'vitro study':ab,ti OR 'vitro studies':ab,ti OR 'human cell*':ab,ti) AND ('titanium ion':ab,ti OR 'titanium ions':ab,ti OR 'ti ion':ab,ti OR 'ti ions':ab,ti OR 'ppm ti':ab,ti) AND ('cellular response':ab,ti OR 'inflammatory response':ab,ti OR 'protein level':ab,ti OR 'gene expression'/exp OR 'gene expression' OR 'transcriptome'/exp OR 'transcriptome' OR 'transcriptomics'/exp OR 'transcriptomics' OR 'transcriptomic*':ab,ti OR 'rna sequencing'/exp OR 'rna sequencing' OR 'rna seq':ab,ti OR 'genome wide':ab,ti OR 'epigenetics'/exp OR 'epigenetics' OR 'epigenomic*':ab,ti OR 'expressed genes':ab,ti OR 'microarray analysis'/exp OR 'microarray analysis' OR 'microarray*':ab,ti OR 'genes expressed':ab,ti OR 'messenger rna'/exp OR 'messenger rna' OR 'mrna*' OR 'rna'/exp OR 'rna' OR 'rnas':ab,ti OR 'microrna'/exp OR 'microrna' OR 'dna damage'/exp OR 'dna damage' OR 'methylation'/exp OR 'methylation' OR 'epigenetic changes':ab,ti OR 'molecular mechanism'/exp OR 'molecular mechanism' OR 'molecular signatures':ab,ti OR 'proteome'/exp OR 'proteome' OR 'proteomics'/exp OR 'proteomics' OR 'proteomic*':ab,ti OR 'protein production'/exp OR 'protein production' OR 'expression*':ab,ti OR 'high throughput sequencing'/exp OR 'high throughput sequencing' OR 'high throughput':ab,ti OR 'genome'/exp OR 'genome' OR 'genomics'/exp OR 'genomics' OR 'genomic*':ab,ti OR 'polymorphism*':ab,ti OR 'histone acetylation'/exp OR 'histone acetylation' OR 'omics'/exp OR 'omics') AND ([english]/lim OR [german]/lim) | 30 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 1c | <p>(‘human cell culture’/exp OR ‘human cell culture’ OR ‘epithelium cell’/exp OR ‘epithelium cell’ OR ‘endothelium cell’/exp OR ‘endothelium cell’ OR ‘tissue culture’/exp OR ‘tissue culture’ OR ‘human tissue, cells or cell components’/exp OR ‘human tissue, cells or cell components’ OR ‘gingiva’/exp OR ‘gingiva*’ OR ‘fibroblast’/exp OR ‘fibroblast*’ OR ‘odontoblast’/exp OR ‘odontoblast’ OR ‘osteoclast’/exp OR ‘osteoclast’ OR ‘osteoblast’/exp OR ‘osteoblast’ OR ‘macrophage’/exp OR ‘macrophage’ OR ‘human monocytes’:ab,ti OR ‘cells cultured’:ab,ti OR ‘vitro study’:ab,ti OR ‘vitro studies’:ab,ti OR ‘human cell*’:ab,ti) AND (‘corrosion product*’:ab,ti OR ‘metal oxide’:ab,ti OR ‘metal particle*’:ab,ti OR ‘metal ion’/exp OR ‘metal ion’ OR ‘metal ions’/exp OR ‘metal ions’ OR ‘metal nanoparticle’/exp OR ‘metal nanoparticle’ OR ‘metal nanoparticles’:ab,ti OR ‘cobalt’/exp OR ‘cobalt’ OR ‘chromium’/exp OR ‘chromium’ OR ‘aluminum’/exp OR ‘aluminum’ OR ‘nickel’/exp OR ‘nickel’ OR ‘vanadium’/exp OR ‘vanadium’ OR ‘implant particle*’:ab,ti) AND (‘titanium’/exp OR ‘titanium’ OR ‘titanium*’:ab,ti OR ‘ti’:ab,ti OR ‘ti3’:ab,ti) AND (‘cellular response’:ab,ti OR ‘inflammatory response’:ab,ti OR ‘protein level’:ab,ti OR ‘gene expression’/exp OR ‘gene expression’ OR ‘transcriptome’/exp OR ‘transcriptome’ OR ‘transcriptomics’/exp OR ‘transcriptomics’ OR ‘transcriptomic*’:ab,ti OR ‘rna sequencing’/exp OR ‘rna sequencing’ OR ‘rna seq’:ab,ti OR ‘genome wide’:ab,ti OR ‘epigenetics’/exp OR ‘epigenetics’ OR ‘epigenomic*’:ab,ti OR ‘expressed genes’:ab,ti OR ‘microarray analysis’/exp OR ‘microarray analysis’ OR ‘microarray*’:ab,ti OR ‘genes expressed’:ab,ti OR ‘messenger rna’/exp OR ‘messenger rna’ OR ‘mrna*’ OR ‘rna’/exp OR ‘rna’ OR ‘rnas’:ab,ti OR ‘microrna’/exp OR ‘microrna’ OR ‘dna damage’/exp OR ‘dna damage’ OR ‘methylation’/exp OR ‘methylation’ OR ‘epigenetic changes’:ab,ti OR ‘molecular mechanism’/exp OR ‘molecular mechanism’ OR ‘molecular signatures’:ab,ti OR ‘proteome’/exp OR ‘proteome’ OR ‘proteomics’/exp OR ‘proteomics’ OR ‘proteomic*’:ab,ti OR ‘protein production’/exp OR ‘protein production’ OR ‘expression*’:ab,ti OR ‘high throughput sequencing’/exp OR ‘high throughput sequencing’ OR ‘high throughput’:ab,ti OR ‘genome’/exp OR ‘genome’ OR ‘genomics’/exp OR ‘genomics’ OR ‘genomic*’:ab,ti OR ‘polymorphism*’:ab,ti OR ‘histone acetylation’/exp OR ‘histone acetylation’ OR ‘omics’/exp OR ‘omics’) AND ([english]/lim OR [german]/lim)</p> | 834 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|---|----------------------|
| 1d | <p>(‘human cell culture’/exp OR ‘human cell culture’ OR ‘epithelium cell’/exp OR ‘epithelium cell’ OR ‘endothelium cell’/exp OR ‘endothelium cell’ OR ‘tissue culture’/exp OR ‘tissue culture’ OR ‘human tissue, cells or cell components’/exp OR ‘human tissue, cells or cell components’ OR ‘gingiva’/exp OR ‘gingiva*’ OR ‘fibroblast’/exp OR ‘fibroblast*’ OR ‘odontoblast’/exp OR ‘odontoblast’ OR ‘osteoclast’/exp OR ‘osteoclast’ OR ‘osteoblast’/exp OR ‘osteoblast’ OR ‘macrophage’/exp OR ‘macrophage’ OR ‘human monocytes’:ab,ti OR ‘cells cultured’:ab,ti OR ‘vitro study’:ab,ti OR ‘vitro studies’:ab,ti OR ‘human cell*’:ab,ti) AND (‘titanium’/exp OR ‘titanium’ OR ‘titanium*’:ab,ti OR ‘ti’:ab,ti OR ‘ti3’:ab,ti) AND (‘actinomyces naeslundii’/exp OR ‘actinomyces naeslundii’ OR ‘aggregatibacter actinomycetemcomitans’/exp OR ‘aggregatibacter actinomycetemcomitans’ OR ‘campylobacter rectus’/exp OR ‘campylobacter rectus’ OR ‘fusobacterium nucleatum’/exp OR ‘fusobacterium nucleatum’ OR ‘porphyromonas gingivalis’/exp OR ‘porphyromonas gingivalis’ OR ‘prevotella intermedia’/exp OR ‘prevotella intermedia’ OR ‘tannerella forsythia’/exp OR ‘tannerella forsythia’ OR ‘treponema denticola’/exp OR ‘treponema denticola’ OR ‘periodontal pathogens’ OR ‘lipopolysaccharide’/exp OR ‘lipopolysaccharide’ OR ‘lipopolysaccharides’:ab,ti OR ‘lps’:ab,ti OR ‘oral bacteria’:ab,ti OR ‘p. gingivalis’:ab,ti) AND (‘cellular response’:ab,ti OR ‘inflammatory response’:ab,ti OR ‘protein level’:ab,ti OR ‘gene expression’/exp OR ‘gene expression’ OR ‘transcriptome’/exp OR ‘transcriptome’ OR ‘transcriptomics’/exp OR ‘transcriptomics’ OR ‘transcriptomic*’:ab,ti OR ‘rna sequencing’/exp OR ‘rna sequencing’ OR ‘rna seq’:ab,ti OR ‘genome wide’:ab,ti OR ‘epigenetics’/exp OR ‘epigenetics’ OR ‘epigenomic*’:ab,ti OR ‘expressed genes’:ab,ti OR ‘microarray analysis’/exp OR ‘microarray analysis’ OR ‘microarray*’:ab,ti OR ‘genes expressed’:ab,ti OR ‘messenger rna’/exp OR ‘messenger rna’ OR ‘mrna*’ OR ‘rna’/exp OR ‘rna’ OR ‘rnas’:ab,ti OR ‘microrna’/exp OR ‘microrna’ OR ‘dna damage’/exp OR ‘dna damage’ OR ‘methylation’/exp OR ‘methylation’ OR ‘epigenetic changes’:ab,ti OR ‘molecular mechanism’/exp OR ‘molecular mechanism’ OR ‘molecular signatures’:ab,ti OR ‘proteome’/exp OR ‘proteome’ OR ‘proteomics’/exp OR ‘proteomics’ OR ‘proteomic*’:ab,ti OR ‘protein production’/exp OR ‘protein production’ OR ‘expression*’:ab,ti OR ‘high throughput sequencing’/exp OR ‘high throughput sequencing’ OR ‘high throughput’:ab,ti OR ‘genome’/exp OR ‘genome’ OR ‘genomics’/exp OR ‘genomics’ OR ‘genomic*’:ab,ti OR ‘polymorphism*’:ab,ti OR ‘histone acetylation’/exp OR ‘histone acetylation’ OR ‘omics’/exp OR ‘omics’) AND ([english]/lim OR [german]/lim)</p> | 217 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 2a | ('animal':ab,ti OR 'animal'/exp OR 'animal' OR 'animal experiment'/exp OR 'animal experiment' OR 'rat'/exp OR 'rat' OR 'rats':ab,ti OR 'mouse'/exp OR 'mouse' OR 'mice':ab,ti OR 'dog'/exp OR 'dog' OR 'dogs':ab,ti OR 'leporidae'/exp OR 'leporidae' OR 'rabbits':ab,ti OR 'in vivo study'/exp OR 'in vivo study') AND ([animal experiment]/lim OR [animal model]/lim) AND ('implant*':ab,ti OR 'tooth implant'/exp OR 'tooth implant' OR 'peri-implant':ab,ti) AND ('tooth'/exp OR 'tooth' OR 'teeth':ab,ti OR 'healthy teeth':ab,ti OR 'oral mucosal tissue*':ab,ti OR 'junctional epithelium'/exp OR 'junctional epithelium' OR 'periodontal connective tissue*':ab,ti OR 'periodontal tissue'/exp OR 'periodontal tissue' OR 'periodontal tissue*':ab,ti OR 'healthy tissue*':ab,ti) AND ('cellular response':ab,ti OR 'inflammatory response':ab,ti OR 'protein level':ab,ti OR 'gene expression'/exp OR 'gene expression' OR 'transcriptome'/exp OR 'transcriptome' OR 'transcriptomics'/exp OR 'transcriptomics' OR 'transcriptomic*':ab,ti OR 'rna sequencing'/exp OR 'rna sequencing' OR 'rna seq':ab,ti OR 'genome wide':ab,ti OR 'epigenetics'/exp OR 'epigenetics' OR 'epigenomic*':ab,ti OR 'expressed genes':ab,ti OR 'microarray analysis'/exp OR 'microarray analysis' OR 'microarray*':ab,ti OR 'genes expressed':ab,ti OR 'messenger rna'/exp OR 'messenger rna' OR 'mrna*' OR 'rna'/exp OR 'rna' OR 'rnas':ab,ti OR 'microrna'/exp OR 'microrna' OR 'dna damage'/exp OR 'dna damage' OR 'methylation'/exp OR 'methylation' OR 'epigenetic changes':ab,ti OR 'molecular mechanism'/exp OR 'molecular mechanism' OR 'molecular signatures':ab,ti OR 'proteome'/exp OR 'proteome' OR 'proteomics'/exp OR 'proteomics' OR 'proteomic*':ab,ti OR 'protein production'/exp OR 'protein production' OR 'expression*':ab,ti OR 'high throughput sequencing'/exp OR 'high throughput sequencing' OR 'high throughput':ab,ti OR 'genome'/exp OR 'genome' OR 'genomics'/exp OR 'genomics' OR 'genomic*':ab,ti OR 'polymorphism*':ab,ti OR 'histone acetylation'/exp OR 'histone acetylation' OR 'omics'/exp OR 'omics') AND ([english]/lim OR [german]/lim) | 348 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|---|----------------------|
| 2b | ('animal':ab,ti OR 'animal experiment'/exp OR 'animal experiment' OR 'rat'/exp OR 'rat' OR 'rats':ab,ti OR 'mouse'/exp OR 'mouse' OR 'mice':ab,ti OR 'dog'/exp OR 'dog' OR 'dogs':ab,ti OR 'rabbit':ab,ti OR 'rabbits':ab,ti OR 'in vivo study'/exp OR 'in vivo study') AND ('implant*':ab,ti OR 'tooth implant'/exp OR 'tooth implant' OR 'peri-implant':ab,ti) AND ('periimplantitis'/exp OR 'periimplantitis' OR 'peri-implantitis':ab,ti OR 'peri implant disease*':ab,ti OR 'peri implant osteolysis':ab,ti OR 'failing dental implant*':ab,ti) AND ('cellular response':ab,ti OR 'inflammatory response':ab,ti OR 'protein level':ab,ti OR 'gene expression'/exp OR 'gene expression' OR 'transcriptome'/exp OR 'transcriptome' OR 'transcriptomics'/exp OR 'transcriptomics' OR 'transcriptomic*':ab,ti OR 'rna sequencing'/exp OR 'rna sequencing' OR 'rna seq':ab,ti OR 'genome wide':ab,ti OR 'epigenetics'/exp OR 'epigenetics' OR 'epigenomic*':ab,ti OR 'expressed genes':ab,ti OR 'microarray analysis'/exp OR 'microarray analysis' OR 'microarray*':ab,ti OR 'genes expressed':ab,ti OR 'messenger rna'/exp OR 'messenger rna' OR 'mrna*' OR 'rna'/exp OR 'rna' OR 'rnas':ab,ti OR 'microrna'/exp OR 'microrna' OR 'dna damage'/exp OR 'dna damage' OR 'methylation'/exp OR 'methylation' OR 'epigenetic changes':ab,ti OR 'molecular mechanism'/exp OR 'molecular mechanism' OR 'molecular signatures':ab,ti OR 'proteome'/exp OR 'proteome' OR 'proteomics'/exp OR 'proteomics' OR 'proteomic*':ab,ti OR 'protein production'/exp OR 'protein production' OR 'expression*':ab,ti OR 'high throughput sequencing'/exp OR 'high throughput sequencing' OR 'high throughput':ab,ti OR 'genome'/exp OR 'genome' OR 'genomics'/exp OR 'genomics' OR 'genomic*':ab,ti OR 'polymorphism*':ab,ti OR 'histone acetylation'/exp OR 'histone acetylation' OR 'omics'/exp OR 'omics') AND ([english]/lim OR [german]/lim) | 234 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 2c | <p>(‘animal’:ab,ti OR ‘animal’/exp OR ‘animal’ OR ‘animal experiment’/exp OR ‘animal experiment’ OR ‘rat’/exp OR ‘rat’ OR ‘rats’:ab,ti OR ‘mouse’/exp OR ‘mouse’ OR ‘mice’:ab,ti OR ‘dog’/exp OR ‘dog’ OR ‘dogs’:ab,ti OR ‘rabbit’:ab,ti OR ‘rabbits’:ab,ti OR ‘in vivo study’/exp OR ‘in vivo study’) AND ([animal experiment]/lim OR [animal model]/lim) AND (‘periimplantitis’/exp OR ‘periimplantitis’ OR ‘peri-implantitis’:ab,ti OR ‘peri implant disease*’:ab,ti OR ‘peri implant osteolysis’:ab,ti OR ‘failing dental implant*’:ab,ti) AND (‘periodontal disease’/exp OR ‘periodontal disease’ OR ‘periodontitis’/exp OR ‘periodontitis’) AND (‘cellular response’:ab,ti OR ‘inflammatory response’:ab,ti OR ‘protein level’:ab,ti OR ‘gene expression’/exp OR ‘gene expression’ OR ‘transcriptome’/exp OR ‘transcriptome’ OR ‘transcriptomics’/exp OR ‘transcriptomics’ OR ‘transcriptomic*’:ab,ti OR ‘rna sequencing’/exp OR ‘rna sequencing’ OR ‘rna seq’:ab,ti OR ‘genome wide’:ab,ti OR ‘epigenetics’/exp OR ‘epigenetics’ OR ‘epigenomic*’:ab,ti OR ‘expressed genes’:ab,ti OR ‘microarray analysis’/exp OR ‘microarray analysis’ OR ‘microarray*’:ab,ti OR ‘genes expressed’:ab,ti OR ‘messenger rna’/exp OR ‘messenger rna’ OR ‘mrna*’ OR ‘rna’/exp OR ‘rna’ OR ‘rnas’:ab,ti OR ‘micrna’/exp OR ‘micrna’ OR ‘dna damage’/exp OR ‘dna damage’ OR ‘methylation’/exp OR ‘methylation’ OR ‘epigenetic changes’:ab,ti OR ‘molecular mechanism’/exp OR ‘molecular mechanism’ OR ‘molecular signatures’:ab,ti OR ‘proteome’/exp OR ‘proteome’ OR ‘proteomics’/exp OR ‘proteomics’ OR ‘proteomic*’:ab,ti OR ‘protein production’/exp OR ‘protein production’ OR ‘expression*’:ab,ti OR ‘high throughput sequencing’/exp OR ‘high throughput sequencing’ OR ‘high throughput’:ab,ti OR ‘genome’/exp OR ‘genome’ OR ‘genomics’/exp OR ‘genomics’ OR ‘genomic*’:ab,ti OR ‘polymorphism*’:ab,ti OR ‘histone acetylation’/exp OR ‘histone acetylation’ OR ‘omics’/exp OR ‘omics’) AND ([english]/lim OR [german]/lim)</p> | 24 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|---|----------------------|
| 3a | <p>('healthy patients':ab,ti OR 'human'/exp OR 'human' OR 'humans':ab,ti OR 'patient'/exp OR 'patient' OR 'patients':ab,ti OR 'individuals':ab,ti) AND ('implant*':ab,ti OR 'tooth implant'/exp OR 'tooth implant' OR 'peri-implant':ab,ti) AND ('healthy teeth':ab,ti OR 'healthy tissue*':ab,ti OR 'natural teeth':ab,ti OR 'periodontal tissue'/exp OR 'periodontal tissue' OR 'periodontal tissue*':ab,ti OR 'teeth':ab,ti OR 'tooth'/exp OR 'tooth' OR 'junctional epithelium'/exp OR 'periodontal connective tissue*':ab,ti OR 'oral mucosal tissue*':ab,ti) AND ('cellular response':ab,ti OR 'inflammatory response':ab,ti OR 'protein level':ab,ti OR 'gene expression'/exp OR 'gene expression' OR 'transcriptome'/exp OR 'transcriptome' OR 'transcriptomics'/exp OR 'transcriptomics' OR 'transcriptomic*':ab,ti OR 'rna sequencing'/exp OR 'rna sequencing' OR 'rna seq':ab,ti OR 'genome wide':ab,ti OR 'epigenetics'/exp OR 'epigenetics' OR 'epigenomic*':ab,ti OR 'expressed genes':ab,ti OR 'microarray analysis'/exp OR 'microarray analysis' OR 'microarray*':ab,ti OR 'genes expressed':ab,ti OR 'messenger rna'/exp OR 'messenger rna' OR 'mrna*' OR 'rna'/exp OR 'rna' OR 'rnas':ab,ti OR 'microrna'/exp OR 'microrna' OR 'dna damage'/exp OR 'dna damage' OR 'methylation'/exp OR 'methylation' OR 'epigenetic changes':ab,ti OR 'molecular mechanism'/exp OR 'molecular mechanism' OR 'molecular signatures':ab,ti OR 'proteome'/exp OR 'proteome' OR 'proteomics'/exp OR 'proteomics' OR 'proteomic*':ab,ti OR 'protein production'/exp OR 'protein production' OR 'expression*':ab,ti OR 'high throughput sequencing'/exp OR 'high throughput sequencing' OR 'high throughput':ab,ti OR 'genome'/exp OR 'genome' OR 'genomics'/exp OR 'genomics' OR 'genomic*':ab,ti OR 'polymorphism*':ab,ti OR 'histone acetylation'/exp OR 'histone acetylation' OR 'omics'/exp OR 'omics') AND ([english]/lim OR [german]/lim)</p> | 1,089 |
| 3b | <p>('healthy patients':ab,ti OR 'human'/exp OR 'human' OR 'humans':ab,ti OR 'patient'/exp OR 'patient' OR 'patients':ab,ti OR 'individuals':ab,ti) AND ('implant*':ab,ti OR 'tooth implant'/exp OR 'tooth implant' OR 'peri-implant':ab,ti) AND ('periimplantitis'/exp OR 'periimplantitis' OR 'peri-implantitis':ab,ti OR 'peri implant disease*':ab,ti OR 'peri implant osteolysis':ab,ti OR 'failing dental implant*':ab,ti) AND ('cellular response':ab,ti OR 'inflammatory response':ab,ti OR 'protein level':ab,ti OR 'gene expression'/exp OR 'gene expression' OR 'transcriptome'/exp OR 'transcriptome' OR 'transcriptomics'/exp OR 'transcriptomics' OR 'transcriptomic*':ab,ti OR 'rna sequencing'/exp OR 'rna sequencing' OR 'rna seq':ab,ti OR 'genome wide':ab,ti OR 'epigenetics'/exp OR 'epigenetics' OR 'epigenomic*':ab,ti OR 'expressed genes':ab,ti OR 'microarray analysis'/exp OR 'microarray analysis' OR 'microarray*':ab,ti OR 'genes expressed':ab,ti OR 'messenger rna'/exp OR 'messenger rna' OR 'mrna*' OR 'rna'/exp OR 'rna' OR 'rnas':ab,ti OR 'microrna'/exp OR 'microrna' OR 'dna damage'/exp OR 'dna damage' OR 'methylation'/exp OR 'methylation' OR 'epigenetic changes':ab,ti OR 'molecular mechanism'/exp OR 'molecular mechanism' OR 'molecular signatures':ab,ti OR 'proteome'/exp OR 'proteome' OR 'proteomics'/exp OR 'proteomics' OR 'proteomic*':ab,ti OR 'protein production'/exp OR 'protein production' OR 'expression*':ab,ti OR 'high throughput sequencing'/exp OR 'high throughput sequencing' OR 'high throughput':ab,ti OR 'genome'/exp OR 'genome' OR 'genomics'/exp OR 'genomics' OR 'genomic*':ab,ti OR 'polymorphism*':ab,ti OR 'histone acetylation'/exp OR 'histone acetylation' OR 'omics'/exp OR 'omics') AND ([english]/lim OR [german]/lim)</p> | 331 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Embase | Results ^a |
|------|--|----------------------|
| 3c | <p>(‘healthy patients’:ab,ti OR ‘human’/exp OR ‘human’ OR ‘humans’:ab,ti OR ‘patient’/exp OR ‘patient’ OR ‘patients’:ab,ti OR ‘individuals’:ab,ti) AND (‘periimplantitis’/exp OR ‘periimplantitis’ OR ‘peri-implantitis’:ab,ti OR ‘peri implant disease*’:ab,ti OR ‘peri implant osteolysis’:ab,ti OR ‘failing dental implant*’:ab,ti) AND (‘periodontal disease’/exp OR ‘periodontal disease’ OR ‘periodontitis’/exp OR ‘periodontitis’) AND (‘cellular response’:ab,ti OR ‘inflammatory response’:ab,ti OR ‘protein level’:ab,ti OR ‘gene expression’/exp OR ‘gene expression’ OR ‘transcriptome’/exp OR ‘transcriptome’ OR ‘transcriptomics’/exp OR ‘transcriptomics’ OR ‘transcriptomic*’:ab,ti OR ‘rna sequencing’/exp OR ‘rna sequencing’ OR ‘rna seq’:ab,ti OR ‘genome wide’:ab,ti OR ‘epigenetics’/exp OR ‘epigenetics’ OR ‘epigenomic*’:ab,ti OR ‘expressed genes’:ab,ti OR ‘microarray analysis’/exp OR ‘microarray analysis’ OR ‘microarray*’:ab,ti OR ‘genes expressed’:ab,ti OR ‘messenger rna’/exp OR ‘messenger rna’ OR ‘mrna*’ OR ‘rna’/exp OR ‘rna’ OR ‘rnas’:ab,ti OR ‘microrna’/exp OR ‘microrna’ OR ‘dna damage’/exp OR ‘dna damage’ OR ‘methylation’/exp OR ‘methylation’ OR ‘epigenetic changes’:ab,ti OR ‘molecular mechanism’/exp OR ‘molecular mechanism’ OR ‘molecular signatures’:ab,ti OR ‘proteome’/exp OR ‘proteome’ OR ‘proteomics’/exp OR ‘proteomics’ OR ‘proteomic*’:ab,ti OR ‘protein production’/exp OR ‘protein production’ OR ‘expression*’:ab,ti OR ‘high throughput sequencing’/exp OR ‘high throughput sequencing’ OR ‘high throughput’:ab,ti OR ‘genome’/exp OR ‘genome’ OR ‘genomics’/exp OR ‘genomics’ OR ‘genomic*’:ab,ti OR ‘polymorphism*’:ab,ti OR ‘histone acetylation’/exp OR ‘histone acetylation’ OR ‘omics’/exp OR ‘omics’) AND ([english]/lim OR [german]/lim)</p> | 292 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Cochrane Library | Results ^a |
|------|--|----------------------|
| | ("cells cultured" OR "endothelial cells" OR "epithelial cells" OR "gingival epithelial cells" OR "tissue cells" OR "tissue culture" OR "vitro studies" OR "vitro study" OR "human cell culture" OR "human cell*" OR "cell, cultured" OR gingiva* OR fibroblast* OR odontoblast* OR osteoclast* OR osteoblast* OR macrophage* OR "human monocytes") -> #1 ("titanium alloy" OR "titanium alloys" OR "titanium dental" OR "titanium dioxide" OR "titanium discs" OR "titanium particle*" OR "titanium substrata" OR "titanium nanoparticle*" OR "implant particle*" OR titanium*) -> #2 | |
| 1a | ("cellular response" OR "inflammatory response" OR "protein level" OR "transcriptome" OR "transcriptomic*" OR "rna seq" OR "genome wide" OR "epigenomics" OR "epigenetics" OR "expressed genes" OR "microarray*" OR "genes expressed" OR "mrna*" OR "RNA" OR "RNAs" OR "microrna*" OR "dna damage" OR "methylation" OR "epigenetic changes" OR "molecular mechanism" OR "molecular signatures" OR "proteome" OR "proteomics" OR "proteomic*" OR "regulated proteins" OR "protein production" OR "expression*" OR "high throughput" OR "genome" OR "genomic*" OR "polymorphism*" OR "histone acetylation" OR "omic" OR "omics") -> #3 #1 AND #2 AND #3 | 27 |
| 1b | ("titanium ion" OR "titanium ions" OR "Ti ion" OR "Ti ions" OR "ppm Ti") -> #4 #1 AND #4 AND #3 | 0 |
| 1c | ("corrosion product*" OR "metal oxide" OR "metal particles" OR "metal ion" OR "metal ions" OR "metal nanoparticles" OR cobalt OR chromium OR nickel OR aluminium OR vanadium OR magnesium OR "implant particle*") -> #5 (titanium* OR "Ti") -> #6 #1 AND #5 AND #6 AND #3 | 24 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Cochrane Library | Results ^a |
|------|---|----------------------|
| 1d | <p>("actinomyces naeslundii" OR "aggregatibacter actinomycetemcomitans" OR "campylobacter rectus" OR "fusobacterium nucleatum" OR "periodontal pathogens" OR "porphyromonas gingivalis" OR "prevotella intermedia" OR "tannerella forsythia" OR "treponema denticola" OR lipopolysaccharide OR LPS OR "oral bacteria" OR "p. gingivalis") -> #7</p> <p>#1 AND #7 AND #6 AND #3</p> | 14 |
| 2a | <p>("animal experiment*" OR "vivo study" OR "vivo studies" OR animal OR rat OR rats OR mouse OR mice OR dog OR dogs OR rabbit OR rabbits) -> #8 (implant* OR "peri-implant") -> #9 (tooth OR teeth OR "healthy teeth" OR "oral mucosal tissue*" OR "junctional epithelium" OR "periodontal connective tissue*" OR "periodontal tissue*" OR "healthy tissue*") -> #10</p> <p>#8 AND #9 AND #10 AND #3</p> | 24 |
| 2b | <p>(periimplantitis OR "peri-implant disease*" OR "peri-implant osteolysis" OR "failing dental implant*") -> #11</p> <p>#8 AND #11 AND #9 AND #3</p> | 4 |
| 2c | <p>("periodontal disease*" OR periodontitis) -> #12</p> <p>#8 AND #11 AND #12 AND #3</p> | 4 |
| 3a | <p>("healthy patients" OR human* OR patients OR individuals) -> #13 ("healthy teeth" OR "healthy tissue*" OR "natural teeth" OR "periodontal tissue*" OR teeth OR tooth OR "junctional epithelium" OR "periodontal connective tissue*" OR "oral mucosal tissue*") -> #14</p> <p>#13 AND #9 AND #14 AND #3</p> | 74 |
| 3b | <p>#13 AND #11 AND #9 AND #3</p> | 22 |

^aNumber of articles found after the first search on 07/01/2021

| PICO | Search string for Cochrane Library | Results ^a |
|------|------------------------------------|----------------------|
| 3c | #13 AND #11 AND #12 AND #3 | 9 |

^aNumber of articles found after the first search on 07/01/2021

C

Publication List

Freitag L, Spinell T, Kröger A, Würfl G, Lauseker M, Hickel R, et al. Dental implant material related changes in molecular signatures in peri-implantitis – A systematic review and integrative analysis of omics in-vitro studies. *Dental Materials* 2023;39:101–13. <https://doi.org/10.1016/j.dental.2022.11.022>.

Spinell T, Kröger A, Freitag L, Würfl G, Lauseker M, Hickel R, et al. Dental implant material related changes in molecular signatures in periimplantitis – A systematic review of omics in-vivo studies. *Dental Materials* 2023;39:1150-1158. <https://doi.org/10.1016/j.dental.2023.09.007>.

T. Spinell, A. Kroeger, L. Freitag, G. Wuerfl, R. Hickel, M. Keschull PR304: Molecular changes in tissue associated with titanium dental implants - a systematic review of omics studies. (2022), E-Poster | Research Presentation. *J Clin Periodontol*, 49: 143-288. <https://doi.org/10.1111/jcpe.13636>

D

Declaration on the Independence of the Work

Based on the doctoral thesis, I composed two papers - published in the journal 'Dental Materials' - which were partially edited by Dr. Annika Kröger and Dr. Thomas Spinell.

I drafted the protocols for the pre-registration of the systematic review and revised them together with Professor Moritz Kebschull and Dr. Annika Kröger. The subsequent duplicate screening of the articles, data extraction and risk of bias analysis was carried out by myself and additionally by a second co-worker (Dr. Thomas Spinell, Gregor Würfl and Dr. Annika Kröger). I devised the underlying methodology for the entire data analysis and conducted the statistical analysis independently using the computer program R. Furthermore, I designed all tables and figures.

E

Affidavit



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Promotionsbüro



Eidesstattliche Versicherung

Freitag, Lena Marie

Name, Vorname

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Titel:

**Dental Implant Material Related Molecular Changes in Peri-implantitis
– A Systematic Review and Integrative Analysis of Omics Studies**

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Maisach, 01.05.2025
Ort, Datum

Lena Marie Freitag
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