# Inflammatory Gene Profile and Particle Presence in Peri-Implant Mucosa: a Pilot Study on 9 Patients

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## ABSTRACT

**Objectives:** The purpose of this pilot study is to compare gene expression in mucosa around dental implants with zirconia abutment to titanium and investigate presence of particles in mucosa samples and on implant heads.
**Material and Methods:** Ten patients with a single implant supported prosthesis connected to zirconia or titanium abutments were invited at the five-year control. A clinical examination and a survey on experience of function and appearance were conducted. A mucosa biopsy taken in close vicinity to the implant were analysed by real-time polymerase chain reaction (qPCR) and presence of particles in a scanning electron microscope/energy-dispersive X-ray spectroscope (SEM/EDX). Cytological smear samples were collected and analysed through inductively coupled plasma mass spectrometry (ICP-MS) to investigate presence of particles on implant heads.
**Results:** In total, 9 patients participated in the study, five with titanium abutments and four with zirconia abutments. All patients were satisfied with function and aesthetics. Titanium and iron particles were detected in mucosa biopsies. The ICP - MS analysis demonstrated presence of zirconia and titanium. Several proinflammatory genes were upregulated in the zirconia abutment group.
**Conclusions:** Around zirconia abutments a slight increase in proinflammatory response and amount of wear particles was seen as compared to titanium. Wear particles of titanium were present in all soft tissue samples, however zirconia particles only in the samples from implants heads/mucosa with zirconia abutments.

**Keywords:** ceramics; dental abutments; dental implants; gene expression; titanium.

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## INTRODUCTION

Titanium implants are widely used in prosthodontic dentistry as a highly successful and reliable rehabilitation option for loss of teeth [1]. Although often successful there are still implants lost due to biological or technical problems [2]. The abutment, a transmucosal connection between implant and the supraconstruction, is usually made of either zirconium dioxide or titanium, whereas cobalt-chromium or titanium alloys is also used [3-5]. The literature on the clinical success and survival of ceramic abutments are scarce, but some small sample studies have proven good results for zirconia abutments in the anterior and premolar regions. In one study following 27 patients for eleven years 96.3% of the zirconia abutments were in function and considered successful [6]. In another study, 23 patients were followed for an equal number of years showing an equally high survival rate for zirconia abutments [7]. However, in contrast our research group found differences in marginal bone loss values when comparing zirconia and titanium abutments on single implants after 5 year in function, with slightly more bone loss around implants with zirconia abutments [8]. Zirconia is 5 times harder than titanium according to Knoop hardness scale [9] and has been claimed to create more wear on the implant head and thereby more misfit compared to titanium abutments [10]. The potential of particle release from interface wear was demonstrated in a laboratory study comparing abutments made of titanium and zirconia where the latter ones expressed both more initial and total wear on the implants [11]. Also, one *in vivo* study comparing tissue samples collected from implants connected to zirconia abutments to samples from contralateral teeth showed significantly higher amount of titanium particles in the peri implant tissues than the control samples, suggesting that particles are produced and released from the interface during functional loading [12]. Titanium particles can trigger proinflammatory responses *in vitro* [13], and titanium particles have been found in tissue samples from implants with marginal bone loss and signs of inflammation [14]. During inflammation, several signal molecules are secreted to attract or differentiate other immune cells, such as interleukin-2 (IL-2), causing proliferation of T-cells and B-cells [15], or interferon-gamma-induced protein 10 (IP-10/CXCL10) attracting immune cells [16]. Furthermore, interleukin-4 (IL-4) regulates T-cell differentiation [17] and tumour necrosis factor-alpha (TNF-α) causes osteoclast differentiation [18]. In dental literature, the biological response to titanium particles in the peri implant mucosa is still unclear, and a unidirectional causal relation is unable to be stated according to two recent systematic reviews [19,20].

Real-time polymerase chain reaction (qPCR) is a reliable method of measuring immune responses in small tissue samples [21,22]. The analysis uses amplification of a specific target DNA strand and allows for quantification of that selected target compared to a reference gene [23]. By adding fluorescence in the DNA amplification cycles the target gene can be monitored in real time, with high sensitivity and specificity [24]. Several articles on aseptic loosening, a bacteria free loosening of medical implants, have used qPCR for analysing inflammatory gene expression in tissues around failed implants [25,26]. Furthermore, previous analyses of possible effect from wear particles have used qPCR analysis to determining cellular responses *in vivo* [27] and *in vitro* [28,29].

The purpose of the pilot study was to investigate and compare two groups with different abutment materials with regards to the inflammatory response in the peri-implant abutment mucosa through qPCR analysis and the presence of particles in the soft tissue and on the implant head. Our hypothesis was that no difference would be seen in regard to gene expression and particle presence.

## MATERIAL AND METHODS

### Patients

Patients were recruited at the 5-year standard clinical examination of their single implant crown at the Brånemark clinic (Västra Götalands region, Göteborg, Sweden). Patients that had received an implant from one specific manufacturer with the same implant macro design and a screw retained crown was selected to allow for a more adequate comparison of the abutment material effect on soft tissue gene response and particle presence. The inclusion and exclusion criteria was based on previous work by our research group focusing on clinical outcomes between zirconia and titanium abutments on single implants after 5 years in function [8].

The inclusion criteria were: externally connected moderately rough Brånemark single implant; screw retained connection of abutments; 5 years in function; and zirconia or titanium abutment.

The exclusion criteria: systemic diseases and severe defects or disease of implant area.

Patients were meeting the inclusion criteria was asked to participate in in this study and when reaching 10 patients this pilot study stopped recruitment.

In total, 5 patients with a zirconia abutment and 5 with a titanium abutment, all on titanium Nobel Brånemark implants (Nobel Biocare AB; Göteborg, Sweden) were included.

### Clinical examination

The clinical examinations were conducted during the dates of 20th of May 2020 to 25th of November, 2022, by two specialist dentists in prosthodontics (V.F.S., S.B.).

The patients were invited to an examination of the implant. The examination involved clinical inspection of the implant mucosa for signs of inflammation, titanium discoloration and esthetical appearance of implant crown as seen by the clinician. Clinical examination of the mucosa with registration of bleeding on probing and pus was furthermore collected. The patients also participated in a short survey regarding their own satisfaction with the construction, chewing ability and aesthetics of the implant crown (Appendix 1).

All participants have read and signed informed consent form. The use of human subjects in this study has been reviewed and approved by the Swedish Ethical Review Authority Dnr: 2019-01899.

### Glass smears

The glass plates 76 x 26 mm (Menzel Gläser GmbH, Braunschweig, Germany) were washed and cleaned with soap and water and wiped with 70% ethanol (HistoLab; Gothenburg, Sweden). The glass plates were treated with 0.5% gelatine (Sigma-Aldrich; St. Louis, Missouri, USA) and 0.05% chromium potassium sulphate (Sigma-Aldrich) by incubating the glass slides during 60 second and thereafter removing the plates and allowing them too completely dry. On three of the patients another kind of glass was used to allow for a higher visualization in a scanning electron microscope (SEM). The glass was an indio-thallium-oxide slides 25 x 25 mm (Technistro; Nagpur, Maharashtra, India). Prior to SEM imaging a glow discharge treatment was performed using GloQube® Plus (Quorum Technologies; Lewes, United Kingdom) at 15 mA for 20 seconds to improve adhesion of cells.

The cytological smear sample for light and SEM was taken with a small micro brush, gently swabbing the mucosa and implant head (Figure 1). The micro brushes were directly smeared on to a glass plate and the plates placed in Karnovsky's solution (Merck; Rahway, New Jersey, USA). The glass plates were rinsed 6 x 5 minutes in pure 0.1M cacodylate buffer Afterwards, the samples were fixated in 1% osmium tetroxide (EMS; Hatfield, Pennsylvania, USA) in 0.1M cacodylate buffer for 5 min at room temperature in the dark. The samples were then washed (3 x fast and 3 x 5 min) with distilled water. After washing, an ascending series of 30% to 100% ethanol solution (Thermo Fisher Scientific; Cambridge, Massachusetts, USA) was used to dehydrate the samples. The ethanol was then replaced with hexamethyldisilane (Sigma-Aldrich) for 2 to 3 minutes and after removal allowed to air dry.

### Mucosa biopsies

The biopsies were harvested from the palatal/lingual side of the implant after removal of the implant crown. Local anaesthesia was conducted by local injection of one ampulla of Citanest® Dental with Octapressin® (Dentsply Sirona Pty Ltd.; Auckland, New Zealand) 30 mg/ml + 0.54 microgram/ml (Dentsply DeTrey GmbH; Konstanz, Germany) and a small tissue biopsy (approximately 2 mm sized) was taken with a 2 mm biopsy punch (Kai Medical GmbH, Solingen, Germany) from the mucosa with near contact to the abutment. The tissue was directly divided with a sterile scalpel (B. Braun Aesculap AG; Tuttlingen, Germany) in to two separate tissue samples. The first sample was placed in RnA later (Thermo Fisher Scientific) and placed in a -80 °C freezer until further testing with real-time polymerase chain reaction (qPCR) analysis. The second sample was fixated in Karnovsky solution (Merck) for sectioning and visualization in light and SEM.

The implant crown was then repositioned according to the manufacturer's description and standard procedure at the clinic.

The tissue samples for microscope analysis were immersed in Karnovsky's solution (Merck) and washed five times with natrium cacodylate buffer (AGAR Scientific; Stansted, United Kingdom).

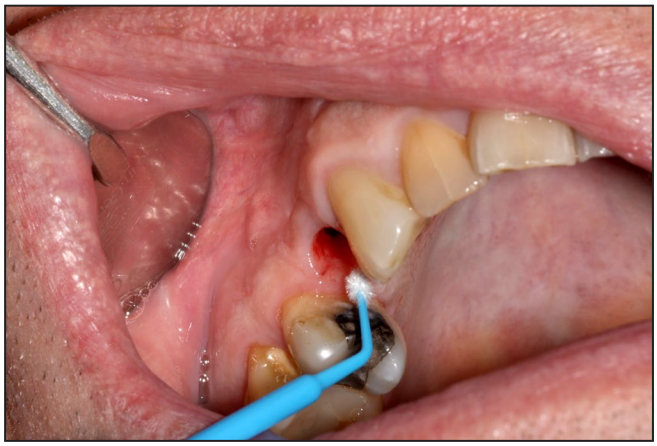

**Figure 1.** Retrieval of samples with micro brush.

The biopsies were post-fixated in osmium tetroxide (EMS) at 4 °C for 1.5 hour in the dark. Thereafter washed with distilled water for 30 minutes and dehydrated in an ascending series of 30 to 100% Ethanol and propylene oxide. After dehydration, the samples were infiltrated with Durcupan™ Resin (Sigma-Aldrich) and left to polymerize for 48 hours at 60 °C. After polymerization, sectioned with a Diatome 45° diamond knife (Diatome, Inc.; Hatfield, Pennsylvania, USA).

**Cellular imaging**

The biopsies and glass plates were placed in a SEM (GeminiSEM 450 - Carl Zeiss Microscopy GmbH; Oberkochen, Germany) connected to a energy-dispersive X-ray (EDX) (Bruker Xflash 6160; Ewing, New Jersey, USA) to allow for finding and characterizing particle debris. The glass plates were placed in an optical microscope Eclipse ME600 (Nikon Co.; Tokyo, Japan) to visualize cells and particle debris.

**ICP analysis**

First a region of interest on the samples was found by using a Raman spectroscopy performed at the Department of Earth Sciences of the University of Gothenburg using a Horiba LabRam HR Evolution Raman spectrometer (Horiba Ltd.; Kyoto, Japan). The analyses were performed with a 532 nm laser after calibration on silicon. Spectra were compared to the Horiba (Horiba Ltd.)/Wiley internal database (KnowItAll software; John Wiley & Sons, Inc., Hoboken, NJ, USA). Inductively coupled plasma-mass spectrometry (ICP-MS) analyses were performed using the laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) system. A NWR 213 laser ablation system (New Wave Research; Fremont, California, USA) is coupled to an Agilent 8800 mass spectrometer (Agilent Technologies Inc.; Santa Clara, California, USA).
The analyses were made during three measurement sessions with identical analytical conditions. All analyses were performed as line scans with a 20 µm round laser beam that moved along the surface of the sample with a scan speed of 1 µm/s. The repetition rate of the laser was set to 5 Hz and the fluence was 3 J/cm$^2$.

**Gene expression analysis**

The samples were collected from the freezer and transported in freezing ice cooler to TATAA Biocenter (Gothenburg, Sweden) for qPCR analysis, immediately unpacked on arrival and stored at -80 ºC until the start of the analysis.

**RNA extraction**

The nine tissue samples from human gum were extracted together with an extraction no-template control (ENTC) consisting of RNase-free water using the extraction kit Total RNA Purification Kit (Cat. No. 17200 - Norgen Biotek Corp., Thorold, Ontario, Canada) according to the manufacturer´s instructions. Before the extraction, the RNA later (Thermo Fisher Scientific, Massachusetts, USA) was removed from the biopsy sample, and lysis buffer was added together with a stainless-steel bead (Ø = 5 mm) to mechanically disrupt the tissue in a Tissuelyser II (Qiagen; Hilden, Germany) for 2 x 5 minutes (25 Hz). The supernatant was transferred to another RNase-free microcentrifuge and the volumes were noted.

**RNA quality control**

The concentration and purity of the extracted RNA were determined using a Little Lunatic spectrophotometer (Unchained Labs, Pleasanton, California, USA). The RNA integrity, reflected in the RNA quality number (RQN) was measured with capillary gel electrophoresis (Fragment Analyzer™ - Agilent Technologies Inc., Santa Clara, California, USA) using the SS Total RNA 15nt Kit (Cat No. DNF-471-33 - Agilent Technologies Inc.).

**RNA normalization and cDNA synthesis**

The extracted RNA samples were normalized to 45 ng/µL, with exception of the samples 6 and 8 (called RNA 6 and RNA 8) which were not normalized, instead, the maximum input volume of the undiluted sample (16.2 ng/µL for samples RNA 6 and 15.2 for samples RNA 8) was used. The samples were reversed transcribed into cDNA using the TATAA GrandScript cDNA Synthesis Kit (Cat. No. A103 - TATAA Biocentre AB; Gothenburg, Sweden), with a mix of poly dT and random primers, included in the RT reaction mix. The reverse transcription was performed in 40 µL reaction volumes with a template volume of 30 µL. Hence, bringing the RNA to 1350 ng/RT reaction, for RNA normalized to 45 ng/µL, 486 ng/RT reaction for sample 6 and 456 ng/RT reaction for sample 8. A reverse transcription no template control (RT-NTC) was included in the RT. The reagents were mixed and run on a T100™ Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, California, USA).

The cDNA was diluted 5 times after the reverse transcription to remove potential inhibitory effect from the extraction and cDNA synthesis part, and to get sufficient volume of cDNA.

**Reference gene screening**

All nine samples were screened for suitable reference genes (RG) using the TATAA human reference gene panel (Cat. No. A101 - TATAA Biocentre), consisting of 12 validated assays. NTCs were included as a control for contamination of reagents and all qPCR reactions were run on the CFX384™ Touch Real-Time PCR System (Bio-Rad Laboratories Inc.) in duplicated 10 µL reactions. The qPCR was performed with TATAA SYBR GrandMaster® Mix (Cat. No. TA01-625 - TATAA Biocenter AB) and the reagents were mixed. Human ValidPrime® (Cat. No. A105P10 - TATAA Biocentre AB) was used to monitor and correct for contaminating gDNA hence duplicates of human gDNA (Cat. No. ChgD24CL, TATAA Biocentre AB) were run on all assays. Interplate Calibrator, IPC (Cat. No. IPC250S - TATAA Biocentre AB), was run on each plate to be able to correct for inter-run differences. A 3-step thermal protocol and melt curve was run as a final step in each qPCR. The detection was performed in the SYBR channel. For all samples, the regression threshold was chosen in the CFX Maestro™ version 2 software (Bio-Rad Laboratories Inc.; California, USA) and qPCR raw data were pre-processed and analysed with GenEx™ software version 7 (MultiD Analyses AB; Göteborg, Sweden) which was also used for ValidPrime® (TATAA Biocenter, Gothenburg, Sweden) correction of gDNA background. The two functions; geNorm and NormFinder in GenEx™ (MultiD Analyses AB) were used to ascertain the most stable reference genes for the sample set.

**Statistical analysis**

Statistical analysis of gene expression was calculated by using the built in Student's t-test in CFX Maestro™ (Bio-Rad Laboratories Inc.).
The significance value level was set to $P < 0.05$.

## RESULTS

**Clinical examination**

One patient (with zirconia abutment) failed to attend the clinical examination due to illness and was not included in the data analysis. Five patients with titanium abutment and four patients with zirconia abutments were examined.
All patients were satisfied with their construction and were pleased with their chewing ability and the aesthetic of the crown. Two patients had visible mucosal inflammation (one zirconia and one titanium). One zirconia patient had visible discoloured mucosa buccally (Figure 2), all others did not. One implant crown (with titanium abutment) was judged as poor esthetical colour all others had excellent or good colour compared to neighbouring teeth (Table 1).

**Microscope analysis**

In samples from patients 1 to 4 several particle-like structures were found around and inside cells when examined with the light microscope (Figure 3). Furthermore, samples showed erythrocytes which could be due to bleeding when removing the implant crown.
SEM/EDX of glass plates from patient 1 and 4 was investigated in the SEM to characterize particles with EDX. Particles of various sized (Figure 4) was found but characterization with EDX was not possible due to poor conductivity, and ICP-MS analysis was chosen instead.
Four biopsy samples from the titanium group and three from the zirconia group were chosen to allow for visualization in the SEM/EDX. The visualization of the biopsies (patients 1 - 2, 4 - 6, 8 - 9) showed several titanium and iron particles, ranging from 200 nm to 1.5 mm. The particles were embedded in the tissues from samples with either titanium or zirconia abutments (Figure 5).

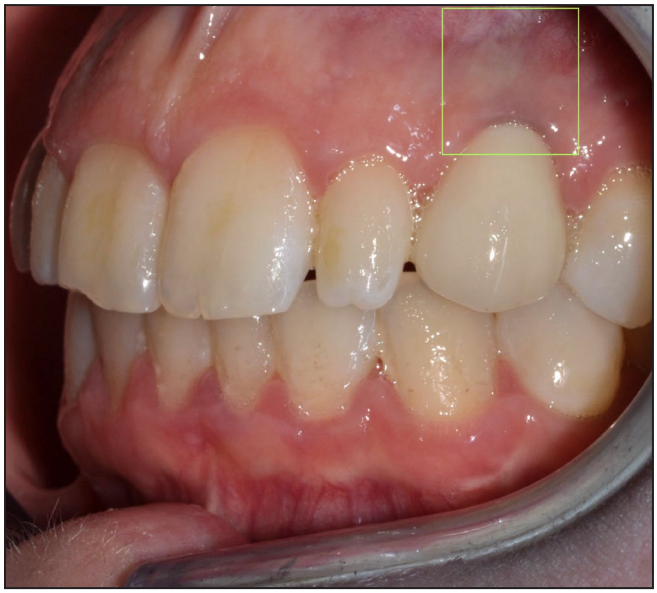

**Figure 2.** Implant shine through thin mucosa of implant crown with zirconia abutment, region of tooth 23.

**Table 1.** Clinical examination: patient questionnaire and clinical findings

| Implant information | | Patient questionnaire | | | | Clinical findings | | |
|---|---|---|---|---|---|---|---|---|
| **Implant position** | **Abutment type** | **Satisfied in general** | **Chewing ability** | **Colour of crown** | **Shape of crown** | **Mucosa** | **Colour** | **Shape** |
| 24 | Titanium | Y | Y | Y | Y | Mucositis | Excellent | Excellent |
| 25 | Titanium | Y | Y | Y | Y | Healthy | Good | Good |
| 14 | Titanium | Y | Y | Y | Y | Healthy | Poor | Good |
| 36 | Titanium | Y | Y | Y | Y | Healthy | good | good |
| 35 | Titanium | Y | Y | Y | Y | Healthy | Good | Good |
| 21 | Zirconia | Y | Y | Y | Y | Healthy | Good | Excellent |
| 21 | Zirconia | Y | Y | Y | Y | Healthy | Excellent | Excellent |
| 23 | Zirconia | Y | Y | Y | Y | Healthy | Good | Good |
| 12 | Zirconia | Y | Y | Y | Y | Mucositis | Good | Good |

Satisfied: Y = yes; N = no; D = don't know.

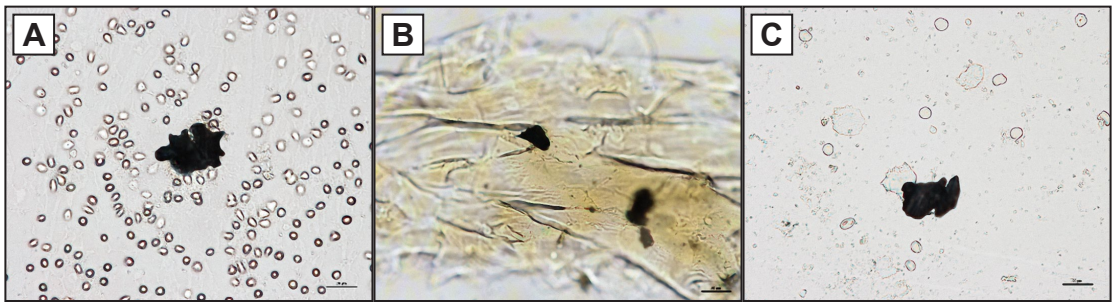


**Figure 3.** Light microscope visualization of particles found in peri-implant mucosa.
Black particles found on glass plate from samples with titanium abutments.
A = swab from implant head and mucosa shows particle around 44 µm and round erythrocytes surrounding it.
B = swab from sample around implant mucosa before detachment of implant crown shows particles embedded in cell, around 18 and 26 µm in size.
C = swab from implant head/mucosa shows particle around 35 µm surrounded with different cells.

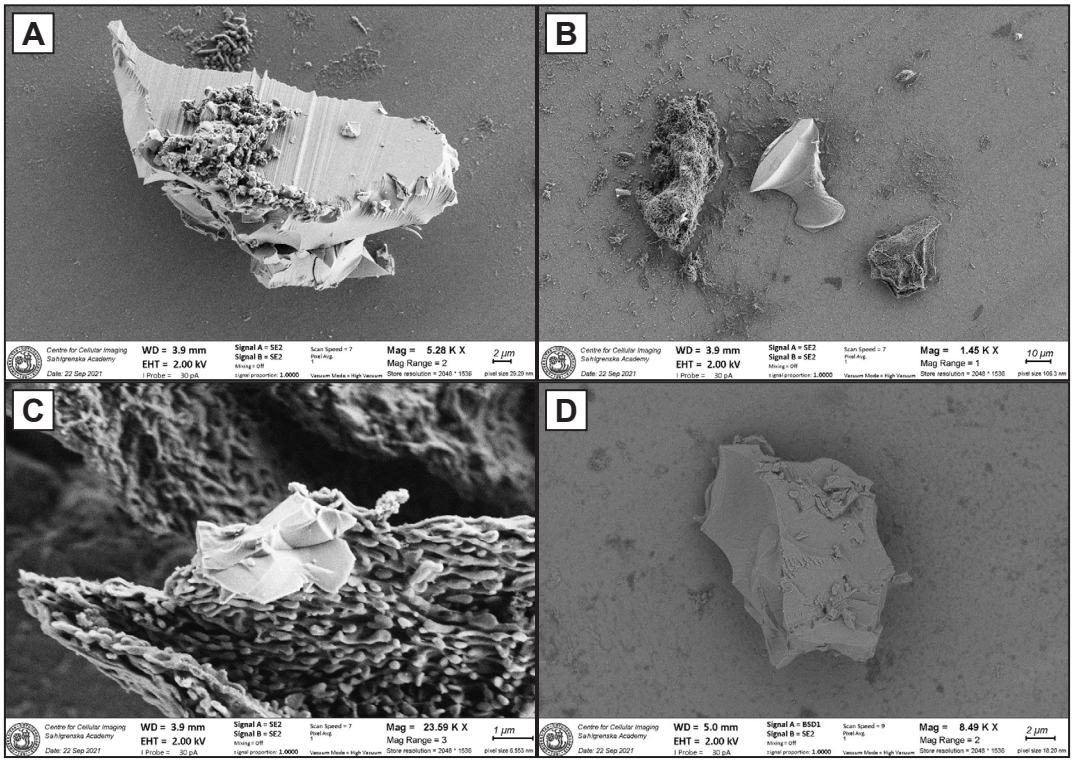


**Figure 4.** Particles found on glass plates from sample 1 (titanium abutment) before detachment of implant crown. Sizes of particles: A = 40 µm; B = 39 µm; C = 3.9 µm; D = 17 µm.

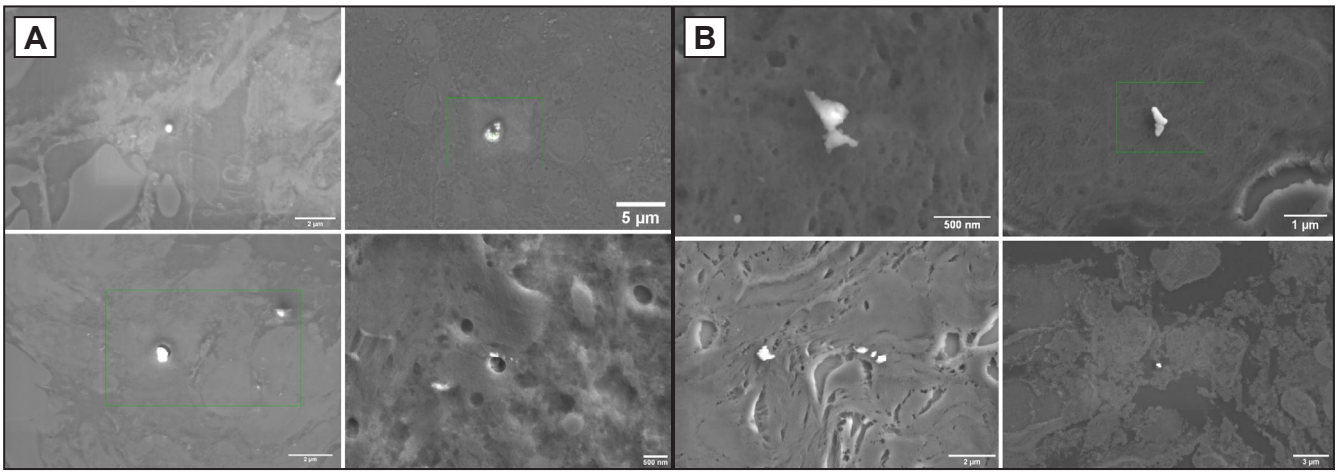


**Figure 5.** Titanium particles in SEM/EDX.
A = samples from patients with titanium abutments; B = samples from patients with zirconia abutments.

The titanium particles identified around zirconia abutments seemed to have sharper edges compared to titanium particles found around titanium abutments. More particles were found in soft tissue samples around implants with zirconia abutments compared to titanium abutments (n: 17/n: 12). Furthermore, iron particles were only found on two patients with titanium abutments. No zirconia particles were found in the tissues when using the SEM analysis.

## ICP-MS analysis

All glass samples were used in the ICP-MS analyses. A region of interest with particulate material was chosen to identify particle debris on the glass slides. On the four samples from implants with zirconia abutments both titanium and zirconia particles were found (Figure 6). On the five samples from implants with titanium abutments no evident signs of particles

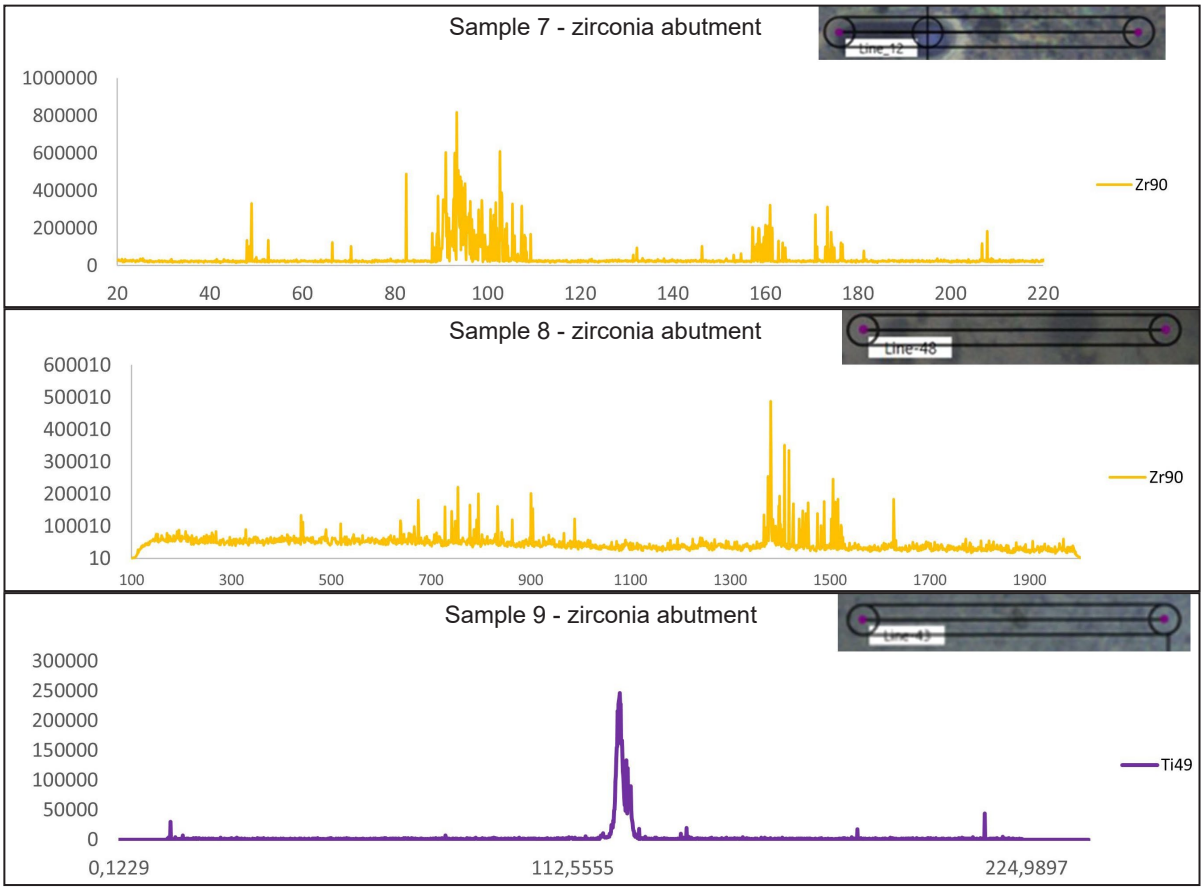


**Figure 6.** ICP-MS graphs of glass plates with swabs taken after detachment of implant crown. The swabs are taken from implant head and surrounding mucosa. Sample 7 to 9 have zirconia abutments. Height and length of line in graph shows detecting a particle with laser.

were found in the ICP-MS analysis. The method did not allow for size measurements of the identified particles in the ICP.

### Gene expression analysis

Several genes were upregulated 2 folds in the zirconia group compared to titanium, CCL2, CCL5, CXCL10, FGF2, IL-2, IFN-γ, neutrophil elastase (NE), receptor activator of nuclear factor kappa-B ligand (RANKL). A few genes were downregulated by 2 folds: colony stimulating factor-3 (CSF-3) and triggering receptor expressed on myeloid cells-1 (TREM1). Statistically significant differences between groups were found for CCL5, CXCl10, IL-2 (upregulated) and TREM1 (downregulated). Table 2 displays gene expression information and Figure 7 - a volcano plot of genes expression.

## DISCUSSION

Seven mucosa samples were used for visualization in SEM, and particles were found in all these tissue samples. The origin of the particles is most likely from the titanium implant or in relevant cases the titanium abutment. The particles can be generated during wear from the implant-abutment interface or when detaching the implant crown as the biopsies were collected. Alrabeah et al. [30] found wear particle release during experimental testing of dental implants. Our research group has previously found similar results when testing experimental loading on dental implants [31]. Another possible origin of wear particles in bone/soft tissues around the implant construction have been suggested to be derived from the implant insertion [32]. The finding of iron particles could further originate from the dental implants as one of the trace element in CP grade 4 titanium is iron [33] or as an artefact during sampling or preparation. Particles found on the implant head could possibly be a result from disassembling implant crowns before testing, and detachment of implant crowns are a common finding in the clinic [34], and hence such particles would detach during reassembling the implant crowns in a clinical milieu.

All patients were satisfied with their implant construction and aesthetics, only one patient with a zirconia abutment showed signs of decolorated mucosa (Figure 2). It is interesting that discoloration was observed in zirconia abutments but not in titanium abutments. Previous studies on mucosal discoloration have indicated that zirconia abutments tend to yield better results compared to titanium [35,36].

**Table 2.** Gene expression analysis

| Gene target | Fold change | P-value | Target regulation |
|---|---|---|---|
| ARG1 | 1.1 | 0.88 | Down regulated |
| MCP1/CCL2 | 2.22 | 0.2 | Up regulated |
| MIP-1α/CCL3 | 1.27 | 0.76 | Down regulated |
| MIP-1β/CCL4 | -1.54 | 0.53 | Down regulated |
| RANTES/CCL5 | 2.82 | 0.05[a] | Up regulated |
| GM-CSF/CSF2 | -1.63 | 0.71 | Down regulated |
| G-CSF/CSF3 | -12.4 | 0.05 | Down regulated |
| IP-10/CXCL10 | 3.82 | 0.02[a] | Up regulated |
| FGF2 | 2.61 | 0.23 | Up regulated |
| IL-10 | -1.29 | 0.66 | Down regulated |
| IL-17 | 1.38 | 0.13 | Down regulated |
| IL-1β | -1.48 | 0.54 | Down regulated |
| IL-1Ra | -1.57 | 0.55 | Down regulated |
| IL-2 | 5.22 | 0.03[a] | Up regulated |
| IL-4 | 1.96 | 0.22 | Down regulated |
| IL-6 | 1.13 | 0.92 | Down regulated |
| IFN-γ | 3.32 | 0.13 | Up regulated |
| MMP-9 | 1.41 | 0.27 | Down regulated |
| NE | 2.49 | 0.48 | Up regulated |
| OPG | 1.26 | 0.69 | Down regulated |
| RANKL | 2.35 | 0.15 | Up regulated |
| TNF-α | 1.73 | 0.33 | Down regulated |
| TRAP | 1.03 | 0.89 | Down regulated |
| TREM1 | -5.44 | 0.04[a] | Down regulated |

[a]Statistically significant at level $P < 0.05$ (Student's t-test).

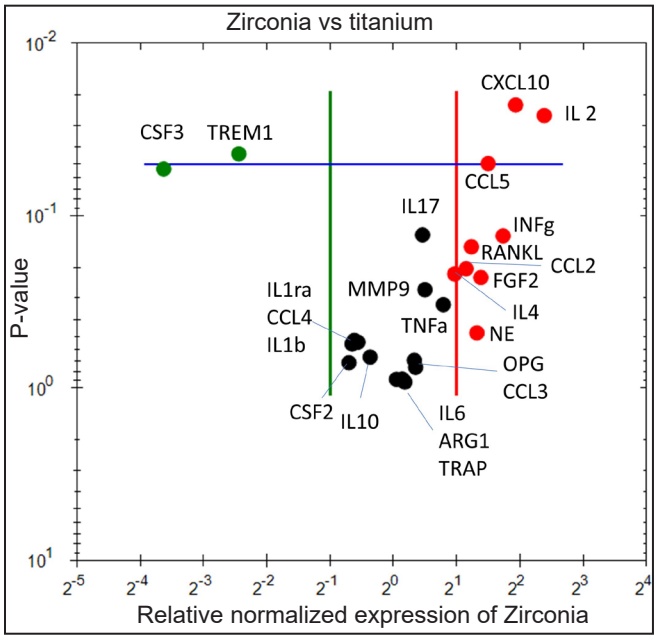


**Figure 7.** Volcano plot illustrating gene expression comparing zirconia biopsy samples to titanium counterparts.
Y-axis P-value (set below 0.05) and X-axis relative expression up/down regulated by two folds.
Red signals upregulated by two-fold, and green down regulated by two-fold. Blue line illustrates significant P-value.

Choosing zirconia abutment on a patient, might be due to lack of buccal bone or thin gingival type prior to implant surgery, as the mucosa tissue thickness can affect shine through of abutment material [35]. In this case, we argue that the implant shine through the mucosa rather than the abutment.

Gene expression analysis showed upregulation of several proinflammatory genes when comparing mucosa from zirconia abutment group to titanium abutments, CCL2 (MCP-1), CCL5 (RANTES), CXCL10 (IP-10), FGF2/FGF basic, IL-2, IFN-γ, NE, RANKL was upregulated by 2 folds. However, only CCL5, CXCL10 and IL-2 showed statistical significance when comparing the groups. CCL5 or RANTES is a chemoattractant for T-cells and monocytes [37] and CXCL10 or IP-10 is regarded as proinflammatory and attracts immune cells [16], IL-2 causes proliferation of T-cells and B-cells [15]. CCL2 or MCP-1 is regarded as proinflammatory and recruits monocytes [38], FGF2 or FGF basic is related to bone homeostasis and skeletal development [39], IFN-γ is regarded as proinflammatory and is an effector of cell mediated immunity [40], NE is stored in the granules of neutrophils and can be released during formation of neutrophil extracellular traps or during inflammatory responses [41]. RANKL is a released by osteocytes an attaches to osteoclast precursor cells which differentiate to mature osteoclasts [42]. TREM1 and CSF-3 were downregulated by 2 folds in zirconia abutment samples compared to titanium abutment samples. Values of TREM1 were also statistically significant difference between the groups. CSF-3 is part of development of neutrophils [43], and TREM1 is a receptor on innate immune cells known to initiate and strengthen inflammation by reacting to infections and DAMPs (damage associated molecular pattern) released during tissue injuries [44].

Using 2-fold upregulation in qPCR studies is a common way to analyse up- and down-regulation of genes, that is the relative difference between control and treated samples [45].

Previous researches on inflammation and zirconia or titanium materials are inconclusive. Obando-Pereda et al. [13] found zirconia particles to generate lower cytokine gene expression in cultured macrophages compared to when cultured with titanium particles. Degidi et al. [46] found larger inflammatory infiltrates around titanium healing caps compared to zirconia healing caps after non submerged healing time of 6 months under non-loading situation. However, in contrast, Barwacz et al. [47] have shown no difference between proinflammatory cytokine expression in peri-implant crevicular fluid after 6 months of use between zirconia and titanium abutments. The outcome of the present pilot study (small sample study) suggests differences in gene expression between mucosa samples from zirconia and titanium abutments. Some proinflammatory genes were upregulated by two folds around zirconia abutment tissue samples and statistically significant, such as CCL5, CXCL10 and IL-2. The difference in gene expression could be related to differences in the amount of wear particles seen between the abutment groups, corresponding well with previous research demonstrating enhanced wear when using zirconia abutments [10]. Susceptible patients might react to titanium wear particles causing pro-inflammatory responses in peri-implant tissues. However, as this study only includes a small sample size a general conclusion on clinical application is not able to be drawn. Future studies on this subject might use other tissue preserving sampling techniques on a large sample size group or use retrieved implants due to implant loss to analyse for presence of metal debris.

## CONCLUSIONS

The result from this pilot study shows that choice of abutment material caused alterations in inflammatory gene expression and presence of particle debris in the adjacent mucosa and on the implant head. Around zirconia abutments a slight increase in proinflammatory response in soft tissues and amount of wear particles was seen. As such, the hypothesis of no difference between the two abutment groups was rejected.

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**Appendix 1.** Patient questionnaire clinical examination

| |
|---|
| Are you in general satisfied with your implant crown?<br>Yes □ No □ Don't know<br><br>Are you satisfied with the chewing ability on your implant crown?<br>Yes □ No □ Don't know<br><br>Are you satisfied with the color of your implant crown?<br>Yes □ No □ Don't know<br><br>Are you satisfied with the shape of your implant crown?<br>Yes □ No □ Don't know |