Prevention and treatment of peri-implant diseases

Cleaning of titanium dental implant surfaces

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Chapter 4

Influence of mechanical instruments on the biocompatibility of titanium dental implant surfaces: a systematic review

A. Louropoulou
D.E. Slot
G.A. van der Weijden

Introduction

The reaction of cells and tissues to biomaterials depends on the material’s properties, surface topography, elemental composition and its behaviour upon contact with the body fluids. Pristine implants, which are made of commercially pure titanium, are covered by a layer of titanium oxide that forms on the surface of the metal within milliseconds of exposure to air, water or other electrolytes (Steinemann, 1998). This oxide layer increases the surface free energy, which facilitates adsorption of biomolecules and subsequent cellular attachment and spreading (Donley & Gillette 1991; Baier, 1988).

Bacterial contamination has been shown to affect cell behaviours and alter the elemental composition of a titanium surface (Kawahara et al. 1998a, 1998b; Mouhyi et al. 2000). Next to bacterial contamination, treatment modalities used to decontaminate the titanium surface can also affect its surface topography and chemical composition (Mouhyi et al. 1998). In addition, it has been shown that some of the instruments used to clean contaminated surfaces may deposit themselves to the treated surfaces, which in turn might disturb cell attachment (Schwarz et al. 2003). Alterations of the titanium surface due to contamination and/or after instrumentation have been shown to induce changes in the oxide layer, resulting in a lower surface energy (Kasemo & Lausmaa 1988). This process appears to impair cell adhesion and affects the biocompatibility of the implant (Baier et al. 1988; Fox et al. 1990; Dmytryk et al. 1990; Mouhyi et al. 1998).

Cleaning of contaminated implant surfaces constitutes an important part in the treatment of peri-implant infections. This review is part of a series of reviews on the effect of mechanical instruments on titanium dental implant surfaces. The cleaning efficacy of these instruments and the surface alterations produced by the instrumentation has been previously published (Louropoulou et al. 2012, 2014). However, a question that arises is which consequences instrumentation has for the attachment of peri-implant tissues. An important goal of the different cleaning procedures is to render the exposed titanium surface biocompatible, with re-osseointegration being the ultimate goal. In addition, if the soft tissue attachment is disrupted during instrumentation, the instrumentation procedure should maintain a surface that is conducive to re-establishment of the soft tissue seal (Kuempel et al. 1995). Therefore, the aim of this review was to systematically evaluate, based on the available evidence, the effect of different mechanical instruments on the biocompatibility of titanium dental implant surfaces.
Materials and Methods

This systematic review was conducted according to the guidelines of Transparent Reporting of Systematic Reviews and Meta-analyses (PRISMA-statement) (Moher et al. 2009).

Focused question
What is the effect of mechanical instruments on the biocompatibility of titanium dental implant surfaces, as assessed by cell responses, compared with untreated (pristine) titanium surfaces?

Search strategy
Three internet sources were used to identify publications that met the inclusion criteria: the National Library of Medicine, Washington, D.C. (MEDLINE-PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL) and EMBASE (Excerpta Medical Database by Elsevier). The search was conducted up to December 2013 and was designed to include any published study that evaluated cell responses on contaminated and non-contaminated titanium dental implant surfaces after treatment with different mechanical instruments. To achieve this goal, a comprehensive search was performed. All reference lists from the selected studies, as well as those of review articles on implants, were manually searched by two reviewers (A.L & G.A.W) for additional papers that met the eligibility criteria. The terms used in the search strategy are presented in Box 1.

Screening and selection
Papers written in English were accepted. Letters, human case reports and reviews were not included in the search. The titles and abstracts were first screened independently by two reviewers (A.L & G.A.W) for eligibility. Following selection, full-text papers were carefully read by the two reviewers. The papers that fulfilled all of the selection criteria were processed for data extraction. Disagreements were resolved by discussion. If disagreements persisted, the judgment of a third reviewer (D.E.S) was decisive. The following eligibility criteria were used:

- Controlled studies, presence of an untreated control
- Titanium surfaces of dental implants or implant components or discs, strips or cylinders simulating such surfaces
- In case of contaminated surfaces, contamination with biofilm grown with a standardised technique, single bacterial species or bacterial products, such as lipopolysaccharide (LPS), or/and calcified deposits
Treatment with mechanical instruments, including curettes and/or scalers, (ultra)sonic instruments, titanium brushes, air abrasives/polishers, rubber cups/points and burs/polishers

Outcome parameters for cell responses, including cell counts, cell growth, cell attachment, cell spreading, cell viability, surface area of cell coverage, and cell morphology

Assessment of heterogeneity

The following factors were evaluated to assess heterogeneity:

- Titanium surfaces
- Surface contamination method, in case of contaminated surfaces
- Cell culture and incubation period
- Treatment performed
- Outcome variables
- Funding

Box 1. Search terms used for PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE. The search strategy was customized according to the database been searched.

\{
\text{\langle Subject\rangle AND \langle Adjective\rangle AND \langle Intervention\rangle}\}

\{\text{\langle Subject: (dental implants [MeSH terms] OR (dental implant OR \{/dental OR oral\} AND implant)\[textword\])\rangle}

\text{AND}

\text{\langle Adjective: (biofilms OR dental plaque OR dental deposits [MeSH terms] OR smooth OR structure OR texture OR roughness OR surface OR biofilm OR plaque index OR dental plaque OR plaque OR dental deposit* OR biocompatibility [textword])\rangle}

\text{AND}

\text{\langle Intervention: (dental scaling OR decontamination OR laser [MeSH terms] OR ultrasonic OR curette OR scaling OR laser OR polishing OR debridement OR curettage OR air abrasion OR air polisher OR cleaning OR instrumentation OR decontamination OR air powder OR bur OR brush [textword]\rangle)\}}
Quality assessment
Two reviewers (A.L & D.E.S) scored the methodological quality of the studies selected for analysis. Assessment of methodological quality was performed as proposed by the RCT checklist from the Dutch Cochrane Centre (2009) and was further extended using quality criteria obtained from the CONSORT statement (Schulz et al. 2010), the Delphi List (Verhagen et al. 1998), the Jadad scale (1996), the ARRIVE guidelines (Kilkenny et al. 2010) and the position papers by Moher et al. (2001) and Needleman (2002). Most of the proposed criteria were combined as described by Louropoulou et al. (2012).

Data extraction and analysis
The data were extracted from the selected papers by two reviewers (A.L & D.E.S). Disagreements were resolved via discussion. If the disagreement persisted, the judgment of a third reviewer (G.A.W) was considered decisive. After a preliminary evaluation of the selected papers, considerable heterogeneity was found in the study characteristics, instruments used, outcome variables and results. Only few studies presented quantifiable data. Consequently, it was impossible to perform valid quantitative analyses of the data or a subsequent meta-analysis. Therefore, a descriptive presentation of the data was adopted.

Grading the ‘body of evidence’
The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system proposed by the GRADE working group was used to grade the collected evidence and to rate the strength of the recommendations (Guyatt et al. 2008).

Results
Search and selection
The PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE searches identified in total, 1,893 unique papers using the specified search terms (Figure 1). The initial screening of the titles and abstracts resulted in eleven full-text papers that met the inclusion criteria. Additional hand-searching of the reference lists from the selected studies and those of review articles did not yield any additional papers. Eleven studies were ultimately processed for data extraction.
Assessment of heterogeneity

Information regarding the study characteristics is provided in Tables 1 and 2. The tables include a short summary of the study design, the results of the selected studies and the authors’ conclusions. After a preliminary evaluation, considerable heterogeneity was found between the selected studies, which precluded any statistical analysis of the data. Therefore, a descriptive manner of data presentation was used. All included studies were in vitro studies. The selected studies could further be divided in two groups: studies evaluating cell behaviours on non-contaminated smooth and structured titanium surfaces after instrumentation with different mechanical instruments and studies evaluating cellular behaviours on smooth and structured titanium surfaces that were contaminated and subsequently cleaned.

Biocompatibility of non-contaminated titanium surfaces after instrumentation

The studies included in this section evaluate the impact of instrumentation on cell responses. Six studies were included in this section. Information on these studies is provided in Table 1.

Four studies (Dmytryk et al. 1990; Kuempel et al. 1995; Shibli et al. 2003; Schwarz et al. 2003) evaluated machined titanium surfaces and three studies (Parham et al. 1989; Rühling et al. 2001; Schwarz et al. 2003) used structured titanium surfaces; SLA (sand-blasted and acid-etched) or TPS (titanium plasma sprayed) surfaces.

Cell cultures and incubation periods varied between the studies. Human or mouse fibroblasts were used in four studies (Parham et al. 1989; Dmytryk et al. 1990; Rühling et al. 2001; Shibli et al. 2003). Schwarz et al. (2003) used osteoblast-like cells (SAOS-2 cells) and Kuempel et al. (1995) rat gingival epithelial cells. The incubation period varied from 24 hours up to 7 days.

Smooth surfaces

Dmytryk et al. (1990) examined the ability of tissue culture fibroblasts to attach and colonize smooth titanium surfaces following instrumentation with curettes of dissimilar composition. The smooth transmucosal extension of IMZ implants was scaled with a stainless-steel, titanium alloy or plastic (acetal plastic) curette and then immersed in a cell suspension of mouse fibroblasts. The number of attached cells was counted at 24 and 72 hours and the implants were then processed for scanning electron microscopy (SEM). At 24 hours, only surfaces scaled with a stainless-steel curette showed a significant reduction in number of attached cells. At 72 hours, significantly fewer cells attached to the surfaces treated with the
stainless-steel and titanium alloy curettes (14.6 ± 2.5, 20.9 ± 4.8, respectively) compared to the untreated control and plastic scaler instrumented surfaces (24.3 ± 2.8, 28.1 ± 6.0, respectively). The greatest reduction in cell attachment was observed on the stainless-steel instrumented surfaces. SEM observations showed that the morphology of cells on titanium-alloy and plastic curette instrumented surfaces was similar to that seen on untreated control surfaces. Fibroblasts on stainless-steel instrumented surfaces tended to show to some extent a rounded morphology and a relatively reduced degree of spreading. The authors attributed the impaired cell attachment after treatment with the stainless-steel curette to an alteration in the surface chemistry produced by the contact of two dissimilar metals.

Kuempel et al. (1995) investigated the ability of epithelial cells to grow on titanium discs simulating the smooth surface of an abutment at the soft tissue interface after instrumentation with stainless-steel, gold-coated and plastic curettes. Rat gingival epithelial cells were used. After 5 days of growth, the epithelial cell surface area coverage (mm²) was measured on photographed specimens using a computer digitizing system. The extent of epithelial cell growth did not differ significantly between the stainless-steel, plastic and untreated control groups (74.4 ± 3.9 mm², 61.2 ± 4.4 mm² and 72.4 ± 3.3 mm², respectively). However, the surfaces treated with the gold-coated curette supported significantly less epithelial growth than the stainless steel and control surfaces (56.7 ± 5.7 mm²), which was thought to be due to changes in the elemental composition of the titanium surface because of damage of the coating of the curette. The slightly reduced epithelial growth on the plastic scaled specimens was attributed by the authors to deposition of particles of the plastic curette on the treated titanium surface.

Treatment of the machined surface of healing abutments with an air powder abrasive system with sodium bicarbonate powder resulted in a reduced proliferation of fibroblasts on the treated surfaces (Shibli et al. 2003). The test group presented a significantly reduced amount of cells (35.31 ± 28.14) as compared to the control group (71.44 ± 31.93) (p = 0.001). This reduced proliferation was attributed by the authors to the release of toxic ions from titanium or the presence of powder particles on the instrumented surfaces. However, no significant differences in cell morphology were found between the groups (p > 0.05), which was considered by the authors a sign of good cell adhesion.

Schwarz et al. (2003) investigated the effects of an ultrasonic scaler (Vector™ system) with a straight carbon fibre tip and polishing fluid (HA particles <10 μm) on the biocompatibility of titanium discs with machined surfaces in cultures of human osteoblast-like cells
(SAOS-2). After an incubation period of 7 days, cells were counted using a reflected light microscope and the cell density per mm² was calculated. The number of attached cells was significantly reduced on the surfaces treated with the Vector™ system compared to the untreated controls (p < 0.001). No differences were observed in the morphology of the cells between test and control groups. The surfaces treated with the Vector™ system showed deposits of the carbon fibre tip used. The authors attributed the reduced cell numbers in the Vector™-treated group to the cytotoxic effect of these fragments from the carbon fibre tip.

**Structured surfaces**

Schwarz et al. (2003) also examined the effect of the same ultrasonic scaler (Vector™ system) on the growth of SAOS-2 cells on rough titanium surfaces. SLA and TPS surfaces were used. The attachment of SAOS-2 cells on the treated surfaces was significantly reduced (p < 0.001), which was, like in the case of machined surfaces, attributed to the cytotoxic effect of the deposits from the used carbon fibre tip. No difference in cell morphology was observed between test and control groups.

Parham et al. (1989) evaluated the attachment of fibroblasts on TPS implant surfaces after treatment with an air powder abrasive system with sodium bicarbonate powder. There were no statistically significant differences in the number of attached cells between treated and control groups. In both treatment groups all specimens were uniformly covered with fibroblasts.

Sometimes the removal of the coating of a rough titanium surface may be necessary, especially when rough implant surfaces become supragingivally exposed. The effect of this treatment on cell behaviour has been addressed in one study (Rühling et al. 2001). These authors investigated the growth of human gingival fibroblasts on the titanium surfaces exposed after the removal of the rough TPS coating using diamond-coated files of different roughness depths. The growth of human gingival fibroblasts on the instrumented surfaces was possible. The cells were ultimately associated to each other, and compared to culture controls on cover glasses, demonstrated good adhesion with strict orientation to the microstructure of the scoring left by instrumentation.

**Biocompatibility of contaminated titanium surfaces after instrumentation**

The studies on contaminated titanium surfaces deal with the impact of both instrumentation and bacterial contamination on cell responses. These studies are more representative of
a clinical situation. Five studies were included in this section. Information on these studies is provided in Table 2.

SLA titanium surfaces were used in the majority of the included studies (John et al. 2014; Schwarz et al. 2009, 2005; Kreisler et al. 2005). Implants with either TPS or machined surfaces were tested in one study by Augthun et al. (1998).

Four studies used an in situ model to contaminate titanium surfaces with supragingival plaque by placing titanium discs in splints in the mouth of volunteers (John et al. 2014; Schwarz et al. 2009, 2005; Augthun et al. 1998), while Kreisler et al. (2005) used contamination with single-species biofilm of Porphyromonas gingivalis.

Cell cultures and incubation periods varied between the studies. Human or mouse fibroblasts were used in two studies (Kreisler et al. 2005 and Augthun et al. 1998, respectively) and osteoblast-like cells (SAOS-2 cells) in three studies (John et al. 2014; Schwarz et al. 2009, 2005). The incubation period varied from 24 hours up to 7 days.

Smooth surfaces
Augthun et al. (1998) examined the growth of mouse fibroblasts on the machined surface of a screw-type implant contaminated with supragingival plaque after cleaning the surface with a plastic curette or an air abrasive system with sodium bicarbonate powder. In the implant treated with the air abrasive, the percentage of viable cells was nearly the same as in the control group (100%). Cell counting showed 570 cells/mm² for the smooth titanium screw and 580 cells/mm² for the control implants. Good cell spreading could also be observed. This was attributed to the cleaning efficacy of the air abrasive, which was found to yield a completely plaque-free surface. In contrast, the cell number/mm² was significantly reduced on the implant treated with the plastic scaler (290 cells/mm²) (p< 0.001). The viable cells showed limited spreading and were located between residual amorphous material and fungus-like structures, which were thought to be due to insufficient cleaning by the plastic curette. However, it should be kept in mind that in this study threaded implants with a machined surface were used. Therefore, these results cannot be directly extrapolated to the smooth surfaces of the healing abutments or transmucosal components.

Structured surfaces
Augthun et al. (1998) also examined the growth of fibroblasts on the TPS surface of a hollow-cylinder implant after using the same instruments. Similar results to the machined surfaces
were observed. The implant treated with the plastic curette showed significantly reduced number of vital cells compared to the implant treated with the air abrasive and the control implant (275 cells/mm², 550 cells/mm² and 580 cells/mm² respectively) (p < 0.001). Reduced cell spreading was observed on the implant treated with the plastic curette.

Kreisler et al. (2005) evaluated the biocompatibility of SLA surfaces contaminated with a suspension of Porphyromonas gingivalis after treatment with an air abrasive system with sodium bicarbonate powder (Kreisler et al. 2005). After treatment, human gingival fibroblasts were incubated on the specimens. The proliferation rate was determined by means of fluorescence activity of a redox indicator which is reduced by metabolic activity related to cellular growth. Proliferation was determined up to 72h. On air powder-treated specimens cell growth was not significantly different from that on sterile specimens.

Schwarz et al. (2009) evaluated the influence of different air-abrasive powders on cell viability at SLA surfaces contaminated with supragingival plaque. Sodium bicarbonate and amino acid glycine powders with different particle sizes were applied on the SLA surfaces. Specimens were incubated with osteoblast-like cells for 7 days and cell viability, expressed as mitochondrial cell activity (MA) (counts/s), was assessed. All treatments resulted in reduced cell viability compared to the non-contaminated and untreated control group (p < 0.001). However, sodium bicarbonate powder resulted in significantly higher viability than the amino acid glycine powders of different particle sizes (p < 0.001). The cell viability in the amino acid glycine group tended to increase with the particle size of the powder, but these differences did not reach statistical significance (p > 0.05). The authors concluded that the SAOS-2 cell viability at contaminated titanium surfaces was mainly influenced by the particle type of the powder and they suggested that a certain amount of surface ablation might improve cell viability at contaminated titanium implants. The reduced cell viability was attributed by the authors to changes in the chemical composition of the titanium surface and in the presence of powder particles on the instrumented surfaces.

Schwarz et al. (2005) evaluated the biocompatibility of titanium discs with SLA surfaces after treatment with an ultrasonic scaler(Vector™ system) with a polyether etherketone fibre tip (PEEK) and a polishing fluid (HA particles<10 μm). The discs were contaminated with supragingival plaque and after treatment they were incubated with osteoblast-like cells for 3 days. Cell viability was measured by means of mitochondrial cell activity (MA) (counts/s). The discs treated with the ultrasonic scaler showed significantly reduced cell viability compared to the non-contaminated and untreated controls (p < 0.001). This reduced biocompatibility...
was attributed to the residual plaque biofilm and to changes of the surface topography (damage) produced by the instrumentation.

John et al. (2014) evaluated the biocompatibility of contaminated SLA surfaces after treatment with a stainless-steel curette or a rotating titanium brush. The biocompatibility of the treated surfaces was evaluated by measuring the viability of SAOS-2 cells by the use of a luminescence assay after 3 and 6 days of incubation. Both treatments resulted in significantly reduced cell viability compared to the non-contaminated and untreated control groups. The cell viability in the stainless-steel curette group was higher than in the corresponding titanium brush group on both dates. However, the differences between these two groups were not statistically significant.

**Quality assessment and GRADE**

The methodological quality assessment of the various studies is presented in Table 3. Of the eleven included studies, seven were considered to have a high potential risk of bias, three were considered to have a moderate risk of bias and one was considered to have a low risk of bias. Eight studies used titanium discs, sheets or platelets, which are considered to be clinically less representative. Five studies provided data regarding randomisation of the treatment, but no study provided data regarding the allocation concealment. In three studies the examiner was blinded to the experimental conditions.

The following criteria were used to rate the quality of the body of evidence and the strength of the recommendations according to GRADE (Guyatt et al. 2008, GRADE working group): potential risk of bias, consistency, directness, precision of the estimate and publication bias. A formal testing for publication bias, as proposed by Egger et al. (1997), could not be used owing to insufficient statistical power because of the limited number of studies evaluating each instrument and the lack of sufficient quantitative data. Five studies reported data regarding the biocompatibility of titanium dental implant surfaces after treatment with an air-powder abrasive system with sodium bicarbonate powder on titanium dental implant surfaces. The available data were rather consistent, indirect and rather precise and had a moderate/high potential risk of bias. As a result, the strength of recommendation was considered to be weak. Three studies reported data regarding the use of stainless-steel curette. The available data were rather inconsistent, indirect and had a moderate to high potential risk of bias. The strength of recommendation is therefore weak. The data reporting on other mechanical instruments were limited, which made grading of the evidence not feasible.
Discussion

The present review focused on the biocompatibility of titanium dental implant surfaces after treatment with different mechanical instruments. This issue has been approached by in vitro experiments.

The reaction of cells and tissues to biomaterials depends on the material's properties, surface topography, elemental composition and its behaviour upon contact with the body fluids. It has been shown that osteoblast-like cells attach more readily to rough surfaces while epithelial cells and fibroblasts prefer smooth and finely textured surfaces (Bowers et al. 1992; Könönen et al. 1992). It has been observed that the surface microstructure can influence epithelial growth and attachment of fibroblasts (Chehroudi et al. 1989, 1990; Brunette & Chehroudi 1999). Therefore, alterations in surface topography may have a selective influence on the attachment of epithelial cells and fibroblasts, thus having an impact on the maintenance or re-establishment of the soft tissue seal around implants after treatment. Kuempel et al. (1995) and Dmytryk et al. (1990) showed that instrumentation of machined titanium surfaces with curettes of dissimilar composition has different impact on epithelial cells and fibroblasts. While instrumentation with stainless-steel curette did not seem to affect the epithelial cell growth, it seems to have an adverse effect on the growth of fibroblasts. Stainless-steel instrumented surfaces showed significantly fewer attached fibroblasts than untreated controlled surfaces (Dmytryk et al. 1990).

One important step in establishing cellular attachment is a chemical attachment between glycoproteins and the titanium oxide layer of the implant (Donley & Gilette 1991). Treatment modalities may sometimes adversely affect the surface topography and/or alter the chemical composition of a titanium surface which in turn may affect the ability of the surface to support cell attachment and spreading. This may be due to contamination of the surface by debris of the instrument deposited on the surface. This seems to be the explanation for the reduced cell numbers observed after treatment of titanium surfaces with a gold-coated curette (Kuempel et al. 1990) or non-metal instruments (Kuempel et al. 1990; Schwarz et al. 2003). The contact of two dissimilar metals could be the reason for the reduced attachment of fibroblasts on implant surfaces instrumented with steel instruments and titanium-alloy curettes compared to non-instrumented control surfaces (Dmytryk et al. 1990).

In clinical situations, the implant surfaces are contaminated with bacterial deposits. Reduced cell growth and cell viability have been observed after treatment of contaminated machined or structured (SLA or TPS) titanium surfaces with either a plastic curette or ultra-
sonic scalers with non-metal tips (Augthun et al. 1998; Schwarz et al. 2005). These results are corroborated to a certain extent by the findings from two other studies that evaluated the viability of osteoblast-like cells cultured on SLA and Osseotite surfaces after treatment with a plastic curette in combination with chlorhexidine gluconate (CHX) (Schwarz et al. 2005, 2006 respectively). In both studies reduced cell viability was observed after treatment with the plastic scaler and CHX compared to the untreated control ($p < 0.001$). Similar results were also reported in a study where an ultrasonic scaler with the same PEEK tip was used in combination with CHX for the treatment of Osseotite surfaces contaminated with plaque (Schwarz et al. 2006). The inability of plastic instruments to restore the biocompatibility of previous contaminated titanium surfaces seems to be due to deposition of debris of these instruments on the titanium surfaces but also to the inability of these instruments to effectively clean especially the structured titanium surfaces (Louropoulou et al. 2014). The alteration of the surface resulting from the cleansing procedure and the biofilm remaining after cleansing seems to be the reason for the reduced cell viability observed after treatment of SLA surfaces with a rotating titanium brush or a steel curette (John et al. 2014). Mouhyi et al. (1998) tested the surface composition of failed and retrieved machined titanium implants after various cleaning procedures. Although some of the tested methods resulted in a macroscopically clean surface, all of them failed to re-establish the original surface elemental composition.

The air-powder abrasive with sodium bicarbonate powder was the treatment modality mostly evaluated and appears to have the least influence on the biocompatibility of titanium surfaces after treatment. When different powders were used on contaminated SLA surfaces, the sodium bicarbonate powder resulted in higher cell viability than amino acid glycine powders of different sizes. This was attributed by the authors to a certain amount of surface ablation (Schwarz et al. 2009). It seems that the more abrasive sodium bicarbonate powder may clean structured SLA titanium surfaces more effectively than the less abrasive amino acid glycine powders, which in turn improves cell viability. Similar results were also observed in the study by Kreisler et al. (2005) that used the same sodium bicarbonate powder on SLA surfaces contaminated with a single bacterial species. However, the use of sodium bicarbonate powder on smooth (machined) titanium surfaces resulted in a significant decrease in the number of attached fibroblasts compared to the untreated control surfaces, although the morphology of the cells was not altered indicating that the adhesion of fibroblasts was not significantly affected (Shibli et al. 2003). This observation may be due to alterations of the surface morphology produced by the abrasive sodium bicarbonate powder (Louropoulou et
al. 2012) or to the presence of powder particles on the instrumented surfaces (Mouhyi et al. 1998). The less abrasive amino-acid glycine powders, which did not affect the surface morphology of smooth titanium surfaces, may affect the biocompatibility of smooth titanium surfaces differently.

Limitations
Reviewing the literature for studies evaluating the biocompatibility of titanium dental implant surfaces after instrumentation with different mechanical instruments in the absence or presence of contamination retrieved limited evidence. From the available ultrasonic and sonic scalers with metal and non-metal tips, only the Vector™ system has been tested. No studies were found testing rubber cups.

Regarding the cells used, fibroblasts were used in the majority of studies (6/10) followed by the osteoblast-like cells (4/10). The behaviour of epithelial cells, which constitute an important component of the peri-implant soft tissue seal, was evaluated in one study only. The use of fibroblast cell lines in the majority of the studies can be explained by the rapid proliferation of the cells (reducing the probability of contamination), the infinite life-span of cells, allowing many repetitions of experiments, and the fact that these cells are easier to grow and maintain. Although it can be assumed that fibroblasts can provide a valid indication as to how mechanical instruments affect the biocompatibility of different titanium surfaces, other cells may respond differently.

Only three studies (Schwarz et al. 2009; Shibli et al. 2003; Parham et al. 1989) provided information regarding the blinding of the examiner to the experimental conditions. The other eight studies either provided no information on this subject or the information was unclear. Although in this kind of in vitro studies it is not common to report on the blinding of the examiners, the authors of this review think that such information is provided.

Summary and Conclusions
Different animal studies indicate that although mechanical debridement of contaminated implant surfaces can result in resolution of the inflammatory lesion, it fails to achieve significant re-osseointegration along the previously contaminated implant surface (Claffey et al. 2008). This means that although the equilibrium between the peri-implant microbiota and the host defence can be re-established leading to an improvement in the clinical parameters,
the implant surfaces are not biocompatible enough to allow direct apposition of new bone and re-osseointegration. The reduced biocompatibility after treatment has been attributed to changes in the surface topography and chemical composition of the titanium surface produced by the instrumentation, but also to the residual biofilm.

In the present study an attempt was made to evaluate the available evidence on the influence of mechanical instruments on the biocompatibility of titanium implant surfaces in a controlled manner. Although the formulation of concrete conclusions is difficult because of the limited available data, it is carefully concluded that:

- Instrumentation may have a selective influence on the attachment of different cells.
- Plastic instruments fail to restore the biocompatibility of contaminated titanium surfaces because of deposition of debris from the instrument on the surface and limited cleansing efficacy, especially in the case of structured titanium surfaces.
- Treatment of contaminated SLA surfaces with either a metal curette or a rotating titanium brush fail to restore the biocompatibility of the surface.
- The air powder abrasive with sodium bicarbonate powder affects the fibroblast-titanium surface interaction after treatment of smooth or structured titanium surfaces the least, even in the presence of plaque contamination. Cell viability on SLA surfaces is influenced by the type of the powder particles used.

**Implications for further research and practical implications**

In this review an attempt was made to evaluate the available evidence on the biocompatibility of titanium implant surfaces after treatment with mechanical instruments. The formulation of concrete conclusions is difficult because of the limited available evidence. However, the cell responses and the mechanism of cellular adhesion on instrumented surfaces require further investigation. The understanding of the biological consequences of instrumentation for the attachment of peri-implant tissues constitute an important first step in understanding the clinical responses and the absence of significant re-osseointegration observed in both animal and human studies. Since the maintenance of the soft tissue seal is of major importance for the long term stability of implants, well-performed *in vitro* and eventually *in vivo* studies are needed to address the effects of instrumentation procedures on cell attachment in order to establish an evidence-based protocol for the use of mechanical instruments in the maintenance of implants and the treatment of peri-implantitis. Especially, epithelial cells deserve further attention as they constitute an important part of this connective tissue seal.
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Declaration of interest
The authors declare that they have no conflict of interest.
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Authors’ contributions:
A. Louropoulou contributed to the conception, design, acquisition, analysis, interpretation of data, drafted the manuscript.
D.E. Slot contributed to the design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.
G.A. van der Weijden contributed to the conception, design, analysis, interpretation of data, critically revised the manuscript for important intellectual content.

All authors gave final approval and agree to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
References


* Studies included in the review
Figure 1. Databases search and literature selection

- Cochrane-CENTRAL: 185
- PubMed-MEDLINE: 1804
- EMBASE: 50

Excluded by title and abstract: 1882

Unique titles & abstracts: 1893

Selected for full-text reading: 11

Excluded after full-text reading: 0

Final Selection: 11

Non-contaminated surfaces: 6
  - Smooth surfaces: 4
  - Rough surfaces: 3

Contaminated surfaces: 5
  - Smooth surfaces: 1
  - Rough surfaces: 5

Included from the reference list: 0
Table 1. Summary of studies evaluating the biocompatibility of non-contaminated titanium surfaces after instrumentation

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Component/Surface(s)</th>
<th>Treatment/Control (n= # of treated surfaces)</th>
<th>Cell culture/Incubation period</th>
<th>Outcome parameter (assessment method)</th>
<th>Authors’ conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwarz et al. (2003)</td>
<td>Titanium discs Machined surface SLA surface TPS surface</td>
<td>- Ultrasonic system with straight carbon fibre tip and polishing fluid (n=16 per surface) - Untreated control (n=16 per surface)</td>
<td>7 days</td>
<td>Counts of attached cells (cell density/ mm2) Cell morphology (SEM)</td>
<td>Statistically significant decrease in the number of cells that attached to the implant surfaces treated with the ultrasonic system compared to control. No difference in cell morphology between test and control.</td>
</tr>
<tr>
<td>Shibli et al. (2003)</td>
<td>Titanium abutments Machined surface</td>
<td>- Air powder abrasive with sodium bicarbonate powder (n=11) - Untreated control (n=11)</td>
<td>24 hours</td>
<td>Counts of attached cells (number of cells on an area of approximately 200 um2) Cell morphology (SEM)</td>
<td>The use of an air-abrasive prophylaxis system on the surface of titanium healing abutments reduced the cells proliferation but did not influence cell morphology.</td>
</tr>
<tr>
<td>Rühling et al. (2001)</td>
<td>Flat titanium specimens TPS surface</td>
<td>- Instrumentation with diamond-coated files (n=5) - Untreated control (n=5)</td>
<td>2 days</td>
<td>Cell growth (SEM)</td>
<td>Cells were associated with one another and, compared to culture controls, demonstrated good adhesion with strict orientation to the microstructure of the scoring left by the instrumentation.</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Component/Surface(s)</td>
<td>Treatment/Control (n= # of treated surfaces)</td>
<td>Cell culture/Incubation period</td>
<td>Outcome parameter (assessment method)</td>
<td>Authors’ conclusions</td>
</tr>
<tr>
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</tr>
<tr>
<td>Kuempel et al. (1995)</td>
<td>Titanium discs</td>
<td>- Stainless-steel curette (n=10)</td>
<td>Ratt gingival epithelial cells</td>
<td>Cell growth (surface of epithelial cell coverage in mm2)</td>
<td>Epithelial surface area coverage on stainless-steel, plastic and control groups did not vary significantly among groups. The gold-coated curette exposed surfaces supported significantly less epithelial growth than the stainless steel and control surfaces.</td>
</tr>
<tr>
<td></td>
<td>Machined surface</td>
<td>- Gold-coated curette (n=10)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- Plastic scaler (n=10)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>- Untreated control (n=10)</td>
<td>5 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dmytryk et al. (1990)</td>
<td>Implant neck</td>
<td>- Stainless-steel curette (n=10)</td>
<td>Mouse fibroblasts</td>
<td>Counts of attached cells (mean number of attached cells)</td>
<td>At 72 hours, stainless steel and titanium-alloy curette instrumented surfaces showed significantly fewer attached cells than untreated control surfaces. Fibroblasts on stainless steel instrumented surfaces tended to show somewhat rounded cell morphology and a relatively reduced degree of spreading</td>
</tr>
<tr>
<td></td>
<td>Machined surface</td>
<td>- Titanium-alloy curette (n=10)</td>
<td>24 and 72 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Plastic curette (n=10)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Untreated control (n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parham et al. (1989)</td>
<td>Implant specimens</td>
<td>- Air powder abrasive with sodium bicarbonate powder (n=6)</td>
<td>Human gingiva fibroblasts</td>
<td>Counts of attached cells (SEM)</td>
<td>Similar numbers of attached cells in control and test. In both groups specimens were uniformly covered by fibroblasts.</td>
</tr>
<tr>
<td></td>
<td>TPS surface</td>
<td>- Untreated control (n=6)</td>
<td>48 hours</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TPS, titanium plasma-sprayed; SLA, sand-blasted and acid-etched; SEM, scanning-electron microscope
Table 2. Summary of studies evaluating the biocompatibility of contaminated titanium surfaces after being mechanically cleaned

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Component/Surface(s)/Contamination</th>
<th>Treatment/control (n= # of treated surfaces)</th>
<th>Cell culture/Incubation period</th>
<th>Outcome parameter (assessment method)</th>
<th>Authors’ conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>John et al. (2014)</td>
<td>Titanium discs SLA surface Contaminated with supragingival plaque by placement of splints in volunteers</td>
<td>- Rotating titanium brush (n=10) - Stainless-steel curette (n=10) - Non-contaminated and untreated control (n=10)</td>
<td>6 days</td>
<td>Osteoblast-like cells</td>
<td>In all treatment groups cell viability was significantly lower compared to the control group. Higher cell viability in the steel curette group than in the titanium brush group.</td>
</tr>
<tr>
<td>Schwarz et al. (2009)</td>
<td>Titanium discs SLA surface Contaminated with supragingival plaque by placement of splints in volunteers</td>
<td>- Air powder abrasive with amino acid glycine or sodium bicarbonate powder (n=128) - Non-contaminated and untreated control (n=8)</td>
<td>7 days</td>
<td>Osteoblast-like cells</td>
<td>In all treatment groups cell viability was significantly lower compared to the control group. Higher cell viability in the sodium bicarbonate group.</td>
</tr>
<tr>
<td>Schwarz et al. (2005)</td>
<td>Titanium discs SLA surface Contaminated with supragingival plaque by placement of splints in volunteers</td>
<td>- Ultrasonic scaler with PEEK tip and polishing fluid (n=20) - Non contaminated and untreated control (n=20)</td>
<td>3 days</td>
<td>Osteoblast-like cells</td>
<td>Treatment with the ultrasonic scaler resulted in significantly lower cell viability compared to control.</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Component/Surface(s)/Contamination</td>
<td>Treatment/control (n= # of treated surfaces)</td>
<td>Cell culture/Incubation period</td>
<td>Outcome parameter (assessment method)</td>
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</tr>
<tr>
<td>Kreisler et al. (2005)</td>
<td>Titanium platelets SLA surface Contaminated with <em>Porphyromonas gingivalis</em></td>
<td>- Air powder abrasive with sodium bicarbonate powder (n=12) - Non contaminated and untreated control (n=12) - Contaminated and untreated control (n=12)</td>
<td>Human gingival fibroblasts 72 hours</td>
<td>Cell proliferation (fluorescence activity of a redox indicator)</td>
<td>Cell growth on the air powder treated specimens was not significantly different from that on non-contaminated and untreated specimens.</td>
</tr>
<tr>
<td>Augthun et al. (1998)</td>
<td>Implants TPS surfaces Machined surface Contaminated with supragingival plaque collected by placement of stents in volunteers</td>
<td>- Plastic scaler (n=2) - Air powder abrasive with sodium bicarbonate powder (n=2) - Non contaminated control (n=2)</td>
<td>Mouse fibroblasts 24 hours</td>
<td>Cell vitality (vital staining)</td>
<td>The percentage of vital cells on implants treated with the air abrasive system was nearly the same as on the control implants. Significantly less vital cell were observed on implant surfaces treated with the plastic scaler.</td>
</tr>
</tbody>
</table>

TPS, titanium plasma-sprayed; SLA, sand-blasted and acid-etched; PEEK, polyether etherketone fibre
Table 3. Methodological quality and risk of bias scores of the selected studies

<table>
<thead>
<tr>
<th>Biocompatibility</th>
<th>Non-contaminated surfaces</th>
<th>Quality criteria</th>
<th>External validity</th>
<th>Internal validity</th>
<th>Statistical validity</th>
<th>Author's estimated risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Author (year)</td>
<td>Representative surface</td>
<td>Validation of the evaluation method</td>
<td>Reproducibility data provided</td>
<td>Sequence generation (randomization)</td>
<td>Concealment allocation</td>
</tr>
<tr>
<td>Non-contaminated surfaces</td>
<td>Shibli et al. (2003)</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>
### Quality criteria

<table>
<thead>
<tr>
<th>Biocompatibility</th>
<th>Contaminated surfaces</th>
<th>Author (year)</th>
<th>External validity</th>
<th>Internal validity</th>
<th>Statistical validity</th>
<th>Author's estimated risk of bias</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>John et al. (2014)</td>
<td>-</td>
<td>+</td>
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<td>High</td>
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<td></td>
<td></td>
<td>Schwarz et al. (2009)</td>
<td>-</td>
<td>+</td>
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<td></td>
<td></td>
<td>Schwarz et al. (2005)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kreisler et al. (2005)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Augthun et al. (1998)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

*+: yes, -: no, ?: not specified/unclear

*: Items used to estimate potential risk of bias

NA: not applicable, visual assessment without scoring of the outcome

1 The authors of the review calculated whether the sample size was adequate by using the Meads’s resource equation (see Louropoulou et al. 2014)