Soft tissue response to zirconia and titanium implant abutments: an in vivo within-subject comparison


Abstract

Aim: To compare the health of the soft tissues towards zirconia and titanium abutments in man, as observed using histological data.

Material and Methods: Twenty patients received two mandibular implants with either a zirconia or titanium abutment (split mouth study design, left-right randomization). After 3 months soft tissue biopsies were prepared for histological evaluation. They were subjected to blind evaluation. The number of blood vessels per surface unit and an inflammation grading scale score ranging from 1 to 4 were determined.

Results: Paired samples from 17 patients were suitable for analysis, 3 with unsevered implant-abutment connections and 14 solely containing soft tissue. All showed a well-keratinized stratified squamous epithelium which was continuous with the barrier (junctional) epithelium that faced the abutment surface. The normal epithelial build-up could be recognized with little signs of inflammation. No statistically significant difference in tissues adjacent to zirconia and titanium abutment surfaces were seen with respect to vascular density (20.5 SD 4.4 and 20.7 SD 3.2) or inflammation grading scale scores (3.2 SD 0.7 versus 3.1 SD 0.7).

Conclusion: No differences in soft tissue health were seen in peri-implant mucosa adjacent to zirconia and titanium abutment surfaces.

Conflict of interest and source of funding

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The authors declare that they have no conflicts of interest.
for all-ceramic crowns. Especially zirconia is promising as it allows a CAD-CAM production process, has a high fracture toughness and favourable light dynamics. White ZrO2 and greyish Ti abutments covered by peri-implant mucosa enhance a different light reflection, which is noticeable to the human eye when the covering mucosa is less than 2 mm thick, which is a common finding in the clinical situation (Jung et al. 2007, van Brakel et al. 2011). As a consequence, soft tissues optically may appear healthier but it is questionable as to whether or not that is biologically the case. A question that so far has been left unanswered on the basis of the limited amount of clinical and biological evidence comparing the two (Myshin & Wiens 2005, Teughels et al. 2006, Klinge & Meyle 2006, Jung et al. 2008, Linkevicius & Apse 2008, Sailer et al. 2009, Zembic et al. 2009, Nakamura et al. 2010). Bone-implant response to Ti and ZrO2 and evaluation of biomechanical properties of these materials have been the main focus of research (Wenz et al. 2008).

The establishment of a stable and healthy perimucosal seal that protects the underlying tissues from the intraoral environment depends heavily upon the adhesion, proliferation and colonization of fibroblastic cells and microorganisms. Abutment surface properties, among which are bio-compatibility (i.e. chemistry), surface topography (i.e. roughness) and surface-free energy are key influencing factors (Quirynen et al. 1993, 1994, Bollen et al. 1996, Rimondini et al. 1997, Abrahamsson et al. 1998, Raspenini et al. 1998, Grossner-Schreiber et al. 2001, Abrahamsson et al. 2002, Hamdan et al. 2006, Rompen et al. 2006, Teughels et al. 2006, Linkevicius & Apse 2008).

Biofilm formation in relation to different substrates is relatively easy to study in humans because it does not require an invasive procedure. ZrO2 discs on a prosthetic device that were worn intra-orally for a day elicited less plaque accumulation than Ti discs. This finding was attributed to the superficial structure of the ZrO2, more specifically, to its electric conductivity (Rimondini et al. 2002, Scarano et al. 2004, Nakamura et al. 2010, Salihoglu et al. 2011). However, from two recent clinical trials involving functional, perimucosal ZrO2 and Ti abutments no difference in bacterial colonization was observed. Short-term differences in clinical parameters regarding the soft tissues adjacent to ZrO2 and Ti abutments were also not apparent in these two studies, involving both partially and fully edentulous subjects (van Brakel et al. 2010–2011, Salihoglu et al. 2011).

The present study focuses on the response and health of the soft tissues towards ZrO2 and Ti implant abutments in man as observed using histological data. The number of blood vessels per surface unit is the primary outcome variable as it is presumed to reflect soft tissue health. It is hypothesized that soft tissues adjacent to ZrO2 and Ti implant abutments exhibit similar counts of blood vessels after 3 months of intraoral function.

Material and Methods

Twenty edentulous patients, 9 men and 11 women, aged between 39 and 76 years (mean, 56.4 years) who were scheduled for two mandibular implants and overdenture treatment were enrolled in the study from January 2008 to January 2009. Inclusion criteria consisted of the following:

- Reasonable to good general health or only mild systemic disease, as expressed by an ASA-score I or II. The American Society of Anesthesiologists (ASA) Score is a global score that assesses the physical status of patients before surgery (de Jong & Abraham-Ingijn 1994);
- Bone height in the mandibular anterior region allowing the placement of 11, 13 or 15 mm screw-type implants. Bone width should be such that implants of 3.5 or 4.0 mm in diameter could be placed;
- No history of previous implant loss, major pathology or irradiation of the (anterior) mandible. The study protocol was reviewed and approved by the medical ethics committee of the University Medical Center, Utrecht and written informed consent was obtained.

Implant installation

Two titanium screw implants (OsseoSpeed™ implants; Astra Tech, Mölndal, Sweden) were placed under local anaesthesia in the region of the former mandibular cuspids. Subjects received antibiotics (Vibramycin, from 1 day pre-operatively 200 mg until 7 days postoperatively, once daily 100 mg) and rinsed with a 0.2% chlorhexidine solution from 2 days pre-operatively until 2 weeks postoperatively, also once daily.

Implant diameter and length of the implants within each subject were similar (Table 1). They were immediately provided with one (experimental) zirconium dioxide (ZrO2) and one titanium (Ti) abutment that functioned as healing abutments (non-submerged implant placement, within-subject comparison, left-right randomization, allocation revealed directly after implant placement). After 2 weeks “tooth” brushing commenced. Subjects were subsequently enrolled in a strict protocol that focused on oral hygiene, but during the experiment the abutments were never professionally cleaned.

Abutments (ZrO2 and Ti)

The (experimental) abutments were especially designed, fabricated and CE-marked for the study and are not commercially available. Ground material for the Ti abutments was titanium grade 4 according to ASTM F-67 and Y-TZP according to ISO 13356 for the ZrO2 specimen (Astra Tech, Figs 1 and 2). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular Ti and ZrO2 abutments by the same manufacturer (i.e. the ZirDesign™ and TiDesign™ abutments; Astra Tech). Surface finish requirements for both abutment types were also similar.

The surface roughness (Ra-value) of the experimental ZrO2 and Ti abutments was measured in random directions at three locations on one specimen of each material by means of contact profilometry. Mean Ra-values were 236 nm (range: 217–255 nm) for the ZrO2 abutment and 210 nm (range: 173–272 nm) for the Ti abutment. Hence, the surface roughness of the materials used was...
prior to histological preparation. All biopsies reached onto the alveolar bone. A suture was placed at the abutment surfaces. Therefore, the surgical approach was changed; especially when at the lingual and buccal side the non-keratinized mucosa was included in the biopsy. Therefore, the surgical approach was changed; a scalpel was used to obtain a biopsy of both the mesial and distal marginal soft tissue adjacent to the ZrO2 and Ti abutment in the remaining 17 patients. These 68 biopsies (17 patients, 2 biopsies, 2 abutments) were triangular shaped, with its base positioned at the abutment surfaces. The base of the triangular biopsies measured 4 mm (the implant diameter) and the distance from the base to the tip of the triangle was 6 mm. All biopsies reached onto the alveolar bone. A suture was placed at the tip of the triangular biopsy as a reference (Fig. 3). All soft tissue specimens were stored in 4% buffered formaldehyde solution for fixation and transportation to the laboratory prior to histological preparation.

Histological preparation and analysis

The specimens were fixed in buffered formaldehyde (pH 7.4) 10% for 24 h and subsequently dehydrated in a graded series of ethanol. The biopsies in which both abutment and soft tissue remained undisturbed were embedded in methylmethacrylate (n = 3 patients, MMA). Following polymerization, non-decalcified, 10-µm thick, longitudinal sections were prepared parallel to the implant length axis using a modified sawing microtome technique and subsequently stained with methylene blue and basic fuchsin (van der Lubbe et al. 1988) (Fig. 4).

The triangular biopsies containing solely soft tissue (n = 17) were embedded in Paraplast paraffin (Klinipath B. V., Duiven, the Netherlands), thereby allowing extended analysis techniques. Sections of 6 µm were cut in the same direction as described above and stained with haematoxylin-eosin (Figs 5–7). To elucidate the presence of blood vessels, staining of their vascular basement membrane was performed using collagen IV (Figs 5–7). Also for this colouring technique three 6 µm-thick sections per biopsy were included.

Light microscopic evaluation of all sections was executed using an optical microscope (Leica MZ12; Leica BV, Rijswijk, the Netherlands) and consisted of a complete morphological qualitative description and quantitative analysis of the tissue response.

For quantitative analysis Regions Of Interest (ROI) measuring 200 µm by 200 µm mm at the soft tissue – abutment interface were defined for two sections per abutment: a mesial and a distal one. The ROI’s were positioned at a distance no further than 100 µm from the interface and a maximum of 500 µm measured from the apical termination of junctional epithelium. The number of blood vessels within each ROI was counted to determine vascular density. Mesial and distal sites were averaged.

In addition, all prepared full specimen were scored with respect to the degree of inflammation on a 4-point scale (Table 1). A higher score represents healthier tissues i.e. less inflammatory response.

Statistical analysis

Since each patient had two materials in his/her mouth, mixed-effect models were chosen to correct for the within patient correlation, with subject as random effect and implant material (ZrO2 or Ti) as fixed effect. The outcome measures were the vascular density adjacent to the implant abutments or the 4-point inflammation scale measurements.

Regarding the former, one may have reservations about computing a mean for ordinal data. However, in histology/pathology it is commonly accepted to interpret histomorpho-

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Table 1. Quantitative histological scoring system for inflammation

<table>
<thead>
<tr>
<th>Response</th>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td>1</td>
<td>Masses of inflammatory cells</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Many inflammatory cells, showing some fibroblasts</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Immature connective tissue, showing fibroblasts with few inflammatory cells</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Normal appearance of connective tissue with few inflammatory cells</td>
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metric grading scales as continuous measures in relatively large, normally distributed populations (Fisher et al. 2002, Hedberg et al. 2005). Most computations were performed in SPSS (SPSS version 17; SPSS inc., Chicago, IL, USA).

For post hoc power analysis G*Power (version 3.1.2) was used (Faul et al. 2007).

Results

Descriptive histology

After histological preparation, paired samples from 17 patients were deemed suitable for further analysis, 3 with unsevered implant-abutment connections and 14 containing solely soft tissue. The process for making the histological specimen in these three patients failed for reasons not known.

With respect to the unsevered specimen (Fig. 4), no difference was observed for the two abutment types. At the abutment site, the collagen fibre bundles were oriented parallel to the abutment surface. The thickness of the epithelium showed normal proportions varying between 0.2 and 0.5 mm. In case of one zirconium oxide abutment a brown coloured exudate could be observed (Fig. 4). Analysis of the paraffin sections revealed the presence of normal mucosa. All specimens showed a well-keratinized stratified squamous epithelium which was continuous with the barrier (junctional) epithelium that faced the abutment surface. The normal epithelial build-up could be recognized; on top the stratum corneum producing keratin and further downwards the stratum granulosum, stratum spinosa and stratum basale. Underneath the epithelium the lamina propria could be recognized. Mostly a normal appearance of connective tissue was observed, showing occasionally immature connective tissue containing fibroblasts with inflammatory cells (Fig. 7a). However, no differences in qualitative histological features between both groups were noted.

Quantitative histology

Based on morphological criteria little inflammation was observed around either abutment types. Scores regarding vascular density and on the inflammation scale did not appear skewed, which was confirmed using non-significant Kolmogorov–Smirnov tests.

The mean vascular density in tissues adjacent to ZrO2 and Ti implant abutments was 20.6 (SD 4.4) and 20.7 (SD 3.2) respectively ($F = 0.07, p = 0.80$). For the 4-point inflammation scale measurements, a mean of 3.1 (SD 0.6) for the ZrO2 implant abutments and 3.2 (SD 0.6) for Ti implant abutments was scored.
man (vascular density: linear mixed model, $\beta \pm SE = 0.27 \pm 1.0$; $p = 0.8$; inflammation scale: linear mixed model, $\beta \pm SE = 0.15 \pm 0.24$; $p = 0.5$).

The effect size of the primary outcome measure is 0.05, which can be considered quite "small" according to popular effect size conventions (Cohen 1977). The power of the test is a mere 5%. An a priori calculation for the required sample size to detect such a small effect size, given a minimum power of 80%, reveals that a population of approximately 3000 people would have to be included.

**Discussion**

Zirconia is increasingly used in percutaneous applications, both as copings for indirect restorations and for implant abutments. When Yttria (Y$_2$O$_3$) is added to ZrO$_2$, favourable properties for biomedical devices are achieved. The ground material is then stabilized in its tetragonal state at room temperature and is commonly referred to as Yttria-stabilized Tetragonal Zirconia Polycrystal (Y-TZP). After sintering it has mechanical properties similar to those of stainless steel (Garvie et al. 1975, Manicone et al. 2007).

Surprisingly little research is available with respect to the soft tissue response towards ZrO$_2$, especially as it compares to Ti. The current study presents human histology, which in general is hard to come by. Histology is important considering the limitations that are associated with the use of common but rather robust parameters that monitor soft tissue health (probing pocket depth, bleeding on probing and recession measurements), subtle differences may better be revealed by means of histomorphometric analysis. Such studies on soft tissue response towards different abutment surfaces have predominantly been performed in animals, but rarely comparing ZrO$_2$ with Ti. Welander et al. observed similar counts of leucocytes, collagen and fibroblasts around abutments made of ZrO$_2$ and Ti in Labrador dogs. Both abutments compared favourably with Au-Pt alloy abutments (Welander et al. 2008). Kohal et al. compared soft tissues adjacent to ZrO$_2$ and Ti implants (not abutments!) in monkeys that functioned with cemented single crowns. After 14 months, biop-

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*(F = 0.40, p = 0.53). Hence, both histological inflammation-related parameters did not reveal statistical significant differences in soft tissue health between mucosa adjacent to ZrO$_2$ and Ti implant abutments in*
ences in anatomical dimensions

may well be due to optical

properties, rather than attributable
to biological evidence.

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References


thase expression, vascular endothelial growth factor expression and proliferative activity in peri-implant soft tissues around titanium and zirconium oxide healing caps. Journal of Peri-
odontology 77, 73–80.

Paul, F., Erdfelder, E., Lang, A. G. & Buchner, A. (2007) GPOWER 3: a flexible statistical power analysis program for the social, behav-


phy, microcomputed tomography, and histology for bone tissue engineering. Tissue Engineering 11, 1356–1367.


de Jong, K. J. & Abraham-Inpijn, L. (1994) A risk-related patient-administered medical ques-
tionnaire for dental practice. International Den-
tal Journal 44, 471–479.


Kohal, R. J., Weng, D., Bachle, M. & Strub, J. R. (2004) Loaded custom-made zirconia and titanium implants show similar osseointegra-
tion: an animal experiment. Journal of Peri-
odontology 75, 1262–1268.

Linkevicius, T. & Apse, P. (2008) Influence of abut-


Quirynen, M., Van der Mei, H. C., Bollen, C. M. L., Scholle, A., Marechal, M., Doornbosch, G. L., Naert, I., Busscher, H. J. & Van Steenber-

and subgingival plaque microbiology. An in vitro study on implants. Journal of Periodontol-
y 65, 162–167.

Rasperrini, G., Maglione, M., Cocconcelli, P. & Simion, M. (1998) In vivo early plaque for-
mation on pure titanium and ceramic abut-


phy and of implant components and connec-


matory cytokine productions of human macro-
phage phagocytising sub-micro titanium parti-
cles by allergy DNA chip (Genopall). BioMedical Materials and Engineering 19, 63–70.

Teughels, W., Van Assche, N., Stiepen, I. & Qui-


Zembic, A., Sañier, I., Jung, R. E. & Hamme-

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Clinical Relevance
Scientific rationale for the study: To date, little information is available regarding the soft tissue response towards zirconia and titanium abutment. This study presents rare human histological data.

Principal findings: Marked differences in soft tissue health, however, were not observed.

Practical implications: Perceived differences in the appearance of soft tissues overlying zirconia and titanium abutments may well be due to optical properties, rather than attributable to biological evi-
dence.