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Abstract

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Randomized controlled clinical pilot study of all-ceramic single-tooth implant reconstructions: clinical and microbiological outcomes at one year of loading

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Key words: ceramic abutments, crowns, dental abutments, dental implants, titanium abutments, zirconia

Abstract
Objective: To test whether or not pink veneering of the submucosal part of zirconia abutments influences clinical, microbiological and histological outcomes of cemented implant-supported single crowns (ISSC).
Materials and methods: A total of 20 patients with one single-tooth implant in the esthetic zone were included. Implants were randomly restored with either pink-veneered zirconia abutments (test group; n = 10) or non-veneered white zirconia abutments (control group; n = 10) and with adhesively cemented all-ceramic crowns. At the 6-month follow-up, soft tissue biopsies were prepared for histological evaluation and microbiological samples were collected around abutments and the respective contra-lateral teeth (in 10 of 20 patients). One year after the initiation of loading, clinical parameters were assessed. Robust linear mixed model and cumulative linked mixed model analyses were performed to investigate the effect of group and time-point on clinical and biological outcomes.
Results: Clinical evaluations revealed stable peri-implant soft tissues in terms of probing pocket depth, but a high BOP index (87.5% control; 80.0% test). No statistically significant differences were observed between the test and control group for any outcome measure (P > 0.05). No major biological complications occurred during the observation period. Histological samples revealed a remarkable degree of inflammation in both groups without clear differences in qualitative histological features. Microbiological evaluation demonstrated a slightly higher bacterial count at implants compared to natural teeth at one year of loading without marked differences between groups.
Conclusion: Limited by a small sample size and a relatively short observation period, pink-veneered zirconia abutments exhibited similar clinical, histological and microbiological outcomes as non-veneered zirconia abutments supporting cemented single crowns.

A natural appearance of the mucogingival architecture around implant-supported reconstructions is one of the major treatment goals especially in esthetically demanding situations. Several factors such as the color or the thickness of the mucosa may influence the display of the peri-implant mucosa (Chang et al. 1999; Furhauser et al. 2005; Park et al. 2007). A large number of studies investigated the influence of the abutment material on the mucosal color (Jung et al. 2007, 2008; Park et al. 2007; van Brakel et al. 2011a,b, Bressan et al. 2011), concluding that ceramic abutments might offer advantages in terms of color compared to the gold standard, metal abutment.

However, in cases with a thin mucosa biotype even with white zirconia abutments, a slight discoloration of the peri-implant tissue could be detected (Bressan et al. 2011). Modifications of the ceramic abutments in terms of color could further improve the esthetic appearance of all-ceramic implant reconstructions and could potentially help to
overcome the current limitations (Ishikawa-Nagai et al. 2007; Happe et al. 2013). Possible modifications may include the use of industrially produced dyed ceramic blanks or a submucosal veneering of abutments. From a biological and microbiological point of view, however, submucosally located veneering ceramic may have a negative impact on the health of the peri-implant tissues. The healing and integration of the oral mucosa to different implant materials was evaluated in a number of preclinical studies and systematic reviews (Abrahamsson et al. 1998, 2003; Linkevicius & Apse 2008; Rompen 2012). An enhanced inflammatory soft tissue reaction and less stable soft tissue dimensions were observed when porcelain-veneered metal abutments and gold abutments were compared to highly sintered Al₂O₃ or titanium abutments (Abrahamsson et al. 1998). In addition, the chemical composition, the abutment design and surface characteristics of different abutment and implant materials may influence the microbial colonization and biofilm formation (Scarno et al. 2004; Teughels et al. 2006; Elter et al. 2008; Welander et al. 2008; Subramani et al. 2009). In vivo and in vitro studies demonstrated that both an increase in surface roughness and of the surface-free energy facilitate biofilm formation on implants and abutment materials (Quirynen et al. 1993, 1996; Quirynen 1994, Quirynen & Bollen 1995, Bollen et al. 1996). The initial adhesion and colonization of microorganisms to an implant surface are considered to have a relevant impact on the pathogenesis of infections related to biomaterials (Quirynen & Bollen 1995). It is speculated that veneering of abutments might lead to a change in surface roughness and could therefore influence the biological reaction of peri-implant tissues.

The aim of this study was to test whether or not modification of the submucosal part of zirconia abutments with pink veneering ceramic influences clinical, microbiological and histological outcomes of single-implant reconstructions during a one-year observation period.

Material and methods

Study design and patient selection

The study was designed as a pilot randomized controlled clinical trial. The treatment protocol as well as detailed inclusion and exclusion criteria were specified in detail in previous publications (Buchi et al. 2014; Thoma et al. 2015). The study protocol was approved by the local ethical committee (KEK-ZH Nr. 2010-0041/5), and written informed consent was obtained before any study procedure was performed. In brief, a total of 20 patients receiving one single-tooth implant (OsseoSpeed, ASTRA TECH Implant System, DENTSPLY Implants, Mölndal, Sweden) in the anterior and premolar area of the maxilla or mandible were enrolled. The 20 implants were restored with implant-borne single-tooth reconstructions using customized zirconia abutments (ATLANTIS Abutment shade 00, DENTSPLY Implants) and all-ceramic crowns (emax®, Ivoclar Vivadent, Schaan, FL). At the time of the final impression, patients were randomly assigned to either receive the test abutment (white zirconia modified with a pink veneering ceramic at the submucosal part) or the control abutment (white zirconia abutment without additional veneering ceramic) using a computer-generated randomization list. Treatment allocation was carried out through sealed envelopes.

Prosthetic protocol and treatment modalities

The customized zirconia abutments were designed and fabricated by means of a CAD/CAM system [ATLANTIS Abutment™; DENTSPLY Implants, Mölndal, Sweden]. The abutments were designed by the company using a cloud-based software [ATLANTIS VAD™ Software; DENTSPLY Implants]. Designs were reviewed and if necessary edited by the dental technician [ATLANTIS™ 3D Editor; DENTSPLY implants] before being produced by the manufacturer and shipped to the dental laboratory at the University of Zurich.

In the test group, the submucosal part of the zirconia abutments was subsequently layered with a pink-shaded veneering ceramic (Creation Zi G2, Klema, Meiningen, Austria) by the dental technician. The ceramic layer had a standardized thickness of 0.5 mm at the level of the abutment – crown marginal shoulder and decreased continuously toward the implant shoulder. In the control group, no abutment modifications were applied. The abutment shoulder was designed to be located circumferentially 1 mm below the mucosal margin. Following the insertion of the abutments, all-ceramic crowns were fabricated by means of the lost-wax technique and the crowns were pressed according to the manufacturer’s instructions [IPS e.max® press, Ivoclar Vivadent, Schaan, FL]. Thereafter, the crowns were adhesively cemented on the abutments using a resin cement (Panavia 21 TC®, Kuraray Medical Inc., Okayama, Japan).

All patients participated in a strict maintenance care program according to their individual needs at the Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich.

Follow-up examination

Follow-up examinations for all patients were performed at baseline (7–10 days after crown insertion), at 6 months and at one year of loading. One blinded single examiner performed all the measurements and analyzed the data.

The following parameters were assessed at baseline and at one year:

Clinical parameters

Plaque control record (PCR) [O’Leary et al. 1972], bleeding on probing (BOP), probing pocket depth (PPD) and the width of keratinized mucosa (KM) were assessed at six sites of the implants by means of a periodontal probe [PCB 12, Hu-Friedy, Leimen, Germany]. Mucosal thickness (MT) around implants sites was assessed to the nearest 0.5 mm at a level of 1 mm apical to the mucosal/gingival margin using an endodontic file and a robber stopper. PCR and BOP were recorded as present [score = 1] or absent [score = 0]. In addition, the mucosal/gingival recession (REC) at the mid-buccal and mid-lingual aspects of implants and contra-lateral teeth was assessed. The distance from the mucosal/gingival margin to the crown margin/cemento-enamel junction was measured to the nearest millimeter by means of a periodontal probe [PCB 12, Hu-Friedy]. The height of the papillae mesial and distal next to the implant crowns and next to the corresponding contra-lateral natural teeth was assessed using the modified papilla Index [Jemt 1997].

At 6 months, microbiological and histological outcome measures were assessed:

Microbiological testing

At the 6-month follow-up, microbiological samples were harvested at the mesial and distal aspects of the implant sites and the corresponding contra-lateral sites using a commercially available assay [micro-IDent® plus, heico Dent, Wolfhausen, Switzerland]. According to the manufacturer’s instructions, the supragingival plaque was first removed with a curette without penetrating into the pocket. The sampling sites were dried with air. For subgingival plaque collection, sterile paper points were inserted into the sulcus for 20 s. The tubes containing subgingival biofilms were forwarded for marker pathogen
analyses (micro-IDent<sup>®</sup>plus, heico Dent). This test uses the polymerase chain reaction (PCR) technique and supplies data on quality and quantity of 11 periodonto-pathogenic species and their affiliation to so-called bacterial complexes. The lower detection limit of this test is 10<sup>4</sup> bacteria.

Harvesting of biopsies
In cases of a sufficient amount of keratinized mucosa, a semi-lunar shaped palatal or lingual biopsy was harvested. For that purpose, a sulcular incision along the abutment was connected to a para-marginal incision [at a distance of 2 mm from the sulcus] at the disto-lingual and mesio-lingual line angles. Para-marginal incisions were performed using a scalpel. The vertical dimension extended from the mucosal margin to the bone crest.

Histological preparation and analyses
The biopsies were fixed in 4% buffered formalin for at least 48 h prior to histological preparation. Thereafter, the specimens were fixated, dehydrated and infiltrated with xylol and paraffin (Paraffin 60 Grad Celsius). Subsequently, specimens were embedded in paraffin and cut into 2- to 5-μm-thick sections using a paraffin microtome (MICROM, Medite GmbH, Dietlikon, Switzerland). All sections were stained with hematoxylin-eosin (HE). Light microscopic evaluation of all sections was performed using an optical microscope (Leica CTR600; Leica, Wetzlar, Germany) at a 200× magnification (see Fig. 1). Evaluations included descriptive histology and a semi-quantitative analysis. For that purpose, three regions of interest [at three levels: sulcular epithelium, junctional epithelium, supracrestal connective tissue] were defined. In each region, a blinded examiner unaware of the treatment allocation analyzed the inflammatory reaction semi-quantitatively using a 4-point scoring scale (1 = low degree of inflammation/low number of inflammatory cells to 4 = very high degree of inflammation/very high number of inflammatory cells).

Statistical analysis
All data were analyzed descriptively calculating mean values and standard deviations or frequency of occurrence (BOP, PCR). For interval scaled data (PPD, KM), a robust linear mixed effects model by robustification of scoring equations using Design Adaptive Scale approach (Koller 2014) was used. Robust statistical methods provide accurate P-values even if some assumptions [e.g., normal distribution] are violated [Erceg-Hurn & Mirosevich 2008]. For BOP and PCR, generalized linear mixed models for binomial data were performed. For the ordinal-scaled variables [microbiological data and modified papilla index], a cumulative linked mixed model was fitted using the R package “ordinal” (http://cran.rproject.org/web/packages/ordinal/ordinal.pdf). In all models, we entered group [white zirconia abutment and pink zirconia abutment] and time-point [baseline and 1 year] as fixed factors and participants as a random factor into the model. For modified papilla index and microbiological data, control vs. implant tooth was additionally entered as a fixed factor into the model. The Kenward-Roger approximation was used to perform F-tests and to estimate P-values for each factor and their interaction in the robust mixed models [Halekoh & Hojsgaard 2014]. For the other models [ordinal and binomial data], P-values were estimated using likelihood ratio tests. Significance levels were set to P < 0.05. All tests were performed using the statistical package R [statistical software R, Foundation for Statistical Computing, Vienna, Austria].

Fig. 1. Representative histological sample showing regions of interest at four levels.
Results

Patients and implants
Twenty patients (13 males, 7 females) with a mean age of 46 ± 15 years (range 21–69 years) were included in the study and examined at baseline, 6 and 12 months. The 10 implants in the test group replaced two incisors and eight premolars. Three implants were located in the mandible and seven in the maxilla. The 10 implants in the control group replaced eight incisors and two premolars. Nine were located in the maxilla and one in the mandible. All 20 implants (OsseoSpeed S 3.5 or 4.0; length 6–15 mm) osseointegrated successfully and could be restored with the final reconstructions as planned. The mean follow-up time for the 6-month examination was 7.7 and 14.8 months for the one-year examination. Between baseline and the one-year follow-up, no implants were lost (100% survival rate), but one crown was lost due to an abutment fracture and later replaced (95% survival rate on the restorative level).

Clinical examination
All data are displayed in Table 1. There were no statistically significant differences in mean PPD values between test and control group at any time-point \((P = 0.169)\). However, a time effect was observed for mean PPD (all implants) being significantly higher at baseline compared to the one-year examination \((P = 0.005)\). Plaque accumulation (PCR) around dental implants slightly increased over time \((P = 0.2)\). At one year, PCR amounted to 50.0% (control) and 30.0% (test), whereas BOP values increased to 87.5% (control) and to 80.0% (test) at one year \((P = 0.003)\). Mean width of keratinized tissue and thickness of the mucosa (MT) at implants sites slightly increased between baseline and the one-year follow-up. The differences for PCR, BOP, KM, MT were not statistically significant between the groups at any time-point \((P > 0.05)\).

At 1 year, only one of the control implants demonstrated a slight recession of 1 mm, whereas all other implants showed a stable mucosal margin. In addition, three patients exhibited recessions at the contra-lateral tooth sites at 1 year. The modified papilla index increased between baseline and 6 months (data not shown), but then slightly decreased to the one-year follow-up (see Table 2). These time effects did not show any statistically significant differences between test and control groups \((P > 0.4)\). In general, implants had lower papilla index scores at the mesial \((P = 0.03)\) and the distal \((P < 0.001)\) aspects compared to contra-lateral natural teeth.

Descriptive histology
Ten of 20 patients agreed for a histological sample at 6 months. Out of these, three belonged to the test group, seven to the control group. In general, the marginal portion of the peri-implant soft tissues appeared to be healthy and to have a regular shape (see Fig. 1). In the most coronal part of the biopsy, the oral epithelium had a regular appearance with all four components, a keratinized stratum corneum with a keratin layer, a stratum granulosum, a stratum spinosum and a stratum basale. Rete pegs had a regular shape, and the underlying connective tissue was well organized with few inflammatory cells. The sulcular epithelium had a thin layer of keratin. No rete pegs were present. The adjacent connective tissue had a regular structure with a low to medium degree of inflammatory cells (macrophages, lymphocytes, granulocytes).

The junctional epithelium did not have a keratin layer and no rete pegs. The underlying connective tissue had a looser structure compared to the sulcular epithelium. The adjacent connective tissue was dominated by the largest amount of inflammatory cells (medium to high degree) compared to all other compartments. More blood vessels were present than in any other compartment.

The supracrestal connective tissue appeared to have a loose structure with relatively thin bundles of collagen fibers. Similar to the compartment of the junctional epithelium, an increased number of blood vessels, but fewer inflammatory cells were observed. A detailed overview on all biopsies and the respective scores in terms of the inflammatory status are given in Table 3.

Microbiological outcomes
For the green complex \((C.\text{spec}., E.\text{corrodens}\text{[Ec]})\), no significant differences were observed between the groups. For the orange-associated complex, two species \((C.\text{rectus}\text{[Cr]}, E.\text{nucleatum}\text{[En]})\) were analyzed revealing a significantly higher bacterial count for \(C.\text{Cr}\) in the test group \((P < 0.04)\). Implant sites (test and control group) had a significantly higher number of \(C.\text{Cr}\) bacteria compared to contralateral teeth \((P = 0.03)\) (see Fig. 2). No significant differences were observed for \(E.\text{En}\) in any of the comparisons.

<table>
<thead>
<tr>
<th>Jemt Score</th>
<th>Baseline</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>1</td>
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<tr>
<td>Papilla mes. (%)</td>
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<td>20</td>
</tr>
<tr>
<td>Contralateral tooth control</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Implant control</td>
<td>0</td>
<td>20</td>
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<tr>
<td>Papilla dis. (%)</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Contralateral tooth control</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Implant test</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1. Clinical outcomes: pocket probing depth (PPD), plaque control record (PCR), bleeding on probing (BOP), mucosal thickness (mean MT), width of keratinized mucosa (KM). SD, Standard deviation; CIL, CII, upper, lower confidence interval. *Statistically significant difference

Table 2. Modified papilla index around implants and contralateral teeth
Table 3. Semi-quantitative histological evaluation: inflammatory reaction in the three compartments. 0, No inflammation; 1, Low degree of inflammation; 2, Medium degree of inflammation; 3, High degree of inflammation; 4, Very high degree of inflammation.

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Group</th>
<th>Sulcular epithelium</th>
<th>Junctional epithelium</th>
<th>Supracrestal connective tissue</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>Control</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
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<td>Control</td>
<td>1</td>
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<td>2</td>
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<td>5</td>
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<td>2</td>
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<tr>
<td>6</td>
<td>Control</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>Test</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Test</td>
<td>3</td>
<td>3</td>
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</tr>
<tr>
<td>13</td>
<td>Test</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

For the other species of the orange complex (Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn), Peptostreptococcus micros (Pm)) and Porphyromonas gingivalis (Pg) from the red complex, no significant differences between the groups and compared to the contralateral teeth were observed. Two species in the red complex (Tannerella forsythia (Tf), Treponema denticola (Td)) showed a significantly higher count around implants compared to natural contra-lateral teeth (Tf P = 0.03; Td P = 0.006), without significant differences between test and control group (Tf P = 1.00; Td P = 0.64) [see Fig. 3a and b]. Actinobacillus actinomycetemcomitans (Aa) was neither detected around implants nor at contra-lateral tooth sites.

Discussion

The present randomized controlled clinical study revealed [i] that veneering of the sub-mucosal part of zirconia abutments did not negatively affect clinical, histological and microbiological outcomes of single-tooth implant crowns and [ii] matured and stable peri-implant tissues with a, in general, slightly higher bacterial count compared to natural teeth at one year of loading.

Biological complications around dental implants encompass any signs of inflammation, bleeding, mucositis, suppuration and soft tissue dehiscences. These complications are reported in the literature very inconsistently and without any standardized methodology [Jung et al. 2012]. Based on a systematic review on the survival rate and the incidence of biological, technical and esthetic complications of single crowns on implants, a cumulative soft tissue complication rate of 7.1% was reported at 5 years [Jung et al. 2012]. In terms of abutment materials, a cumulative 5-year rate for biological complications of 5.2% was reported for ceramic and of 7.7% for metal abutments [Sailer et al. 2009]. The present clinical study on 20 patients with zirconia abutment did not show any severe biological complications such as suppuration or bone loss ≥2 mm, but reported one implant (control group) with a soft tissue recession of 1 mm. This resulted in a soft tissue complication rate of 5% at one year of loading. This relatively low rate of soft tissue complications is supported by mean PPD values (<3 mm) around dental implants that, in general, decreased over time indicating matured and stable peri-implant tissues.

BOP values were relatively high at all time-points compared to control teeth. In addition, BOP values increased from baseline (33% of the implants in the test group and 40% of the implants in the control group) to the one-year follow-up (85% and 80%). This observation may be attributed to false-positive results or increased clinical signs of peri-implant inflammation. The geometry of the abutments having a concave design and the obtained maturation of the peri-implant tissues at the one-year follow-up may have contributed to a difficult accessibility for probing and a higher probing force than recommended. It has been shown in a previous study that probing around implants demonstrated a higher sensitivity compared to probing around teeth. The use of 0.25 N probing force induced epithelial bleeding in the absence of soft tissue infection around oral implants. Therefore, a threshold pressure of 0.15 N was recommended to be applied to avoid false-positive observations [Gerber et al. 2009]. A further explanation for relatively high BOP values might be a poorer oral hygiene. PCR scores increased from 20% to 22% at baseline to 30–50% at one year and might in part explain higher BOP values. All patients were placed in an individual maintenance program and attended dental hygiene sessions at least once a year. These hygiene sessions followed immediately after the follow-up visits for this study. As oral hygienic habits may have deteriorated between two recall intervals, a higher inflammatory status and poorer oral hygiene might be expected.

The study design included the use of adhesively cemented reconstructions. As the location of the crown margin was 0.5–1 mm below the mucosal margin, cement excess located submucosally could have been undetected. Several studies and a systematic review have shown that residual excess cement is common after crown cementation on implants [Agar et al. 1997; Linkevicius et al. 2013; Vindasiute et al. 2015]. In
these studies, excess cement was detected independent of the technique used for cementation and irrespective of the implant location, despite meticulous cleaning of the abutment/crown after cementation. Clinically, excess dental cement has been associated with signs of bleeding on probing, suppuration, mucositis and peri-implantitis [Wilson 2009; Korsch et al. 2015]. These clinical signs of inflammation were attributed to the fact that cement retains microbes, and the rough surface of the cement inhibits the removal of the microorganisms. Taking into account all these disadvantages of cemented reconstructions, one might speculate that high BOP scores (clinical signs) could be caused by undetected cement remnants. Moreover, these clinical signs should be reflected in histological and microbiological outcome measures.

Histological data obtained in the present study were based on 10 biopsies (7 control group/3 test group). A remarkable degree of inflammation could be confirmed at 6 month in either group without clear differences in qualitative histological features. With the exception of one histological sample showing a low degree of inflammation, nine of the obtained soft tissue histological samples showed a medium degree of inflammation in the three different compartments. Inflammatory cells were mostly present within and adjacent to the junctional epithelium. The presence of inflammatory cells in the junctional epithelium surrounding implants appears to be a result of a microbial challenge in adjacent sulcus areas as reported by preclinical studies [Berglundh et al. 1992; Ericsson et al. 1995; Abrahamsson et al. 1998; Zitzmann et al. 2002]. Plaque accumulation around the marginal portion of the abutments may have led to an inflammatory reaction in this area. Two samples in the present study additionally harbored a marked inflammatory cell infiltrate in the subepithelial connective tissue compartment lateral to the abutment/implant junction. This inflammatory cell infiltrate may be explained by the host response to bacterial migration through the microgap between the abutment and fixture part of the implant [Quirynen & van Steenberge 1993].

Clinical studies documenting the soft tissue response to zirconia abutments involving histological outcome measures are scarce [van Brakel et al. 2012]. Data provided mainly report on clinical and periodontal parameters, most often comparing titanium and zirconia abutments. Based on these studies, both types of abutments appear to elicit a similar soft tissue response [Sailer et al. 2009; van Brakel et al. 2011a,b; Zembic et al. 2013].

A marked qualitative or quantitative difference in the bacterial colonization of veneered and non-veneered zirconia abutment surfaces was not observed in the present study. Only, Campylobacter rectus (Cr) showed slightly higher bacterial counts in the test group compared to the control group. However, Tannerella forsythia (Tf) and Treponema denticola (Td) were more frequently detected around implants compared to contra-lateral natural sites. In vitro and in vivo studies have shown that healthy peri-implant pockets are characterized by high proportions of Gram-positive oral streptococci and rods, a low number of Gram-negative species and low detection frequencies for bacteria associated with periodontitis [Adell et al. 1986; Lekholm et al. 1986; George et al. 1994; van Winkelhoef et al. 2000, Furst et al. 2007; Kocar et al. 2010]. Anaerobic putative periodontal pathogens such as Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf) and Prevotella intermedia (Pi) are often isolated from failing sites [Mombelli et al. 1987, 1995; Quirynen & Teughels 2003], but can also be detected around stable sites [Papaioannou et al. 1996; Sboronde et al. 1999, Leonhardt et al. 2002, 2003]. These species are most likely part of the normal resident microbiota of most individuals. In a clinical study, the relative amount and not the presence of these pathogens were linked with peri-implantitis [Hultin et al. 2002]. In contrast to sites with peri-implantitis, none of the healthy implant sites reached a $10^6$ threshold level for individual key pathogens. It was concluded that other factors at the patient level (systemic and genetic factors, host susceptibility) were involved in the survival and failure of implants [Hultin et al. 2002].
Since in the present study, the overall counts of key pathways were below the reported threshold value of 10^6, one might assume that all implant sites were stable and healthy and not influenced by the veneering of zirconia abutments.

Limitations applying to the present study predominantly include a small sample size and a relatively short observation period. The study was designed as a pilot randomized controlled clinical trial. Sample size calculation was not possible, as there was no former known clinical trial evaluating a similar study design and similar outcome measures. Longer-term follow-up examinations focusing again on biological outcomes will be needed in the future.

Conclusion
Veneering of the submucosal part of zirconia abutments did not negatively affect clinical, histological or microbial outcomes of cemented implant-supported single crowns compared to non-veneered zirconia abutments. Limitations, however, include a small sample size and a relatively short observation period.

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