Zirconia and titanium implant abutments.

Clinical, microbiological, histological and optical aspects

Colofon

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Zirconia and titanium implant abutments.
Clinical, microbiological, histological and optical aspects

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microbiologische, histologische en optische aspecten
(met een samenvatting in het Nederlands)

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Ralph van Brakel

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Prof. dr. M.S. Cune
Prof. dr. C. de Putter

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Chapter 1

General introduction and aims of the study

Over the years, most research in implant dentistry has focused on the interaction of the endosseous implant body and its surrounding hard tissues and on the biomechanics of the implant(system) (Wenz et al, 2008). The dental community has gradually managed to improve implant concepts and techniques, both surgically and restoratively, as well as on implant design. Shape and size of the dental implants converged to more uniform cylindrical and threaded patterns, rendering blade and subperiostal frame designs as non-suited. A major breakthrough was the shift from rather smooth, machined titanium (*Ti*) surfaces to rougher, surface-treated microstructures during the 2 last decades. As a consequence, when implants are placed today, there is confidence among dental professionals and among the public that they will generally osseointegrate and that they can bear functional loads for a long period of time.

Traditionally, implants have been applied in edentulous situations, serving as abutments for fixed bridges or providing support and retention to complete dentures in case of functional denture problems. When their application in partially edentulous situations emerged, this opened formidable restorative opportunities, but also new challenges. Whereas in former times emphasis was on the restoration of function, today good esthetic results are expected as well. Dental implant therapy has evolved into a well-researched, predictable treatment option, but with respect to the aesthetic outcome, steps for improvement can still be made. A better understanding of soft tissue response to permucosal implant materials and to implant-abutment designs can help to improve the mimicking of not just the natural tooth ('the white'), but especially the supporting soft tissues ('the pink'). The architecture, contour, surface texture and colour of the permucosal tissue are important determinants of the appearance of the final restoration.

Titanium has been the 'gold standard' material for implant abutments (Figure 1a). Clinical studies have proven that its application yields a stable and healthy permucosal seal, thus

Figure 1a. Titanium stock abutment mounted on a titanium implant (Astra Tech Implant System, OsseoSpeed™ implant, Dentsply Implants, Mölndal, Sweden).

Figure 1b. Zirconia stock abutment mounted on a titanium implant.

protecting the underlying tissues from the intraoral environment. This is assumed to depend upon the adhesion, proliferation and colonization of fibroblastic cells and micro-organisms to the implant abutment material. Abutment surface properties, among which are biocompatibility (i.c. chemistry), surface topography (i.c. roughness) and surface-free energy are key influencing factors (Quirynen et al, 1993; Quirynen et al, 1994; Bollen et al, 1996; Rimondini et al, 1997; Abrahamsson et al, 1998; Rasperini 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 and Apse, 2008).

In addition to *Ti*, the use of high-strength ceramics in implant dentistry has increased. Yttria partially stabilized tetragonal zirconia polycrystalline (also known as 'zirconia', Y-TZP or ZrO_2 is especially promising because of its high fracture toughness and favourable light dynamics (Figure 1b). Though not very translucent, it has gained popularity as implant abutment material, especially in regions of high aesthetic demand (Watkin and Kerstein, 2008). The white colour of ZrO_2 is considered aesthetically advantageous because gray *Ti* abutments may hamper the aesthetic outcome, resulting from blue-gray shimmering of the material. This is especially true for cases with thin overlying mucosal tissues. This may cause a noticeable colour difference with the gingival tissues of neighbouring teeth (Park et al, 2007) (Figure 2).

Figure 2a. CAD-CAM fabricated zirconia abutment (Atlantis®), mounted on a titanium implant.

Figure 2b. All ceramic, veneered restoration on a CAD-CAM zirconia abutment (Atlantis®), mounted on a titanium implant.

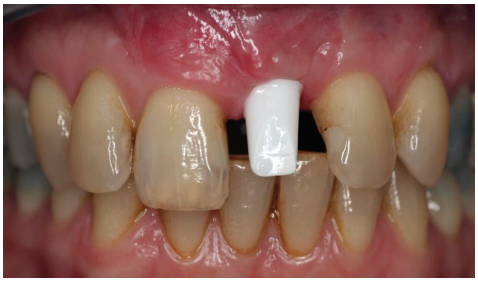

Figure 2c. Intraoral situation. Zirconia abutment in situ.

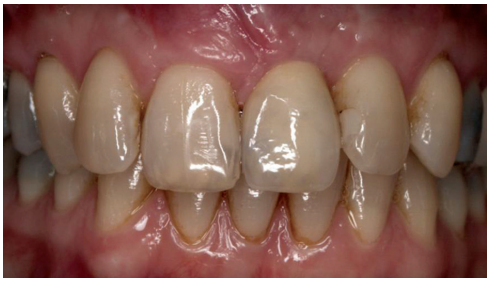

Figure 2d. Cemented restoration after 2 years of function.

For ZrO_2 to be able to compete with *Ti* as ground material for implant abutments, amongst economical and biomechanical factors, it should be at least as biologically friendly and yield similar or better aesthetic results, when applied in humans. Despite its common application, there is only a limited amount of evidence available comparing the two materials regarding clinical, microbiological, histological and optical aspects in man. These aspects form the backbone of this PhD thesis.

Specific aims of the study: questions of investigation

1. Do ZrO_2 and *Ti* abutment surfaces exhibit similar clinical characteristics of peri-implant soft tissue health and microbiological features during the first 3 months? Chapter 2
2. Do soft tissues adjacent to ZrO_2 and *Ti* implant abutments exhibit similar histological aspects after 3 months of intraoral function? Chapter 3
3. Can clinical and microbiological parameters reflect soft tissue health around implant abutments when compared to histological observations, under relatively healthy conditions? Chapter 4
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Chapter 2

Early bacterial colonization and soft tissue health around zirconia and titanium implant abutments: an in vivo study in man

R van Brakel
MS Cune
AJ van Winkelhoff
C de Putter
JW Verhoeven
W van der Reijden

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Abstract

Aim: To compare the early bacterial colonization and soft tissue health of mucosa adjacent to Zirconia (ZrO_2) and Titanium (*Ti*) abutment surfaces in vivo.

Materials and Methods: Twenty edentulous subjects received 2 endosseous mandibular implants. The implants were fitted with either a ZrO_2 or a *Ti* abutment (non-submerged implant placement, within-subject comparison, left-right randomisation). Sulcular bacterial sampling and the assessment of probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were performed at 2 weeks and 3 months post-surgery. Wilcoxon matched-pairs, sign-rank tests were applied to test differences in the counts of 7 marker bacteria and the clinical parameters that were associated with the ZrO_2 and *Ti* abutments, at the 2 observation time points.

Results: Zirconia and titanium abutments harboured similar counts of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Peptostreptococcus micros, Fusobacterium nucleatum and Treponema denticola at 2 weeks and 3 months. Healthy clinical conditions were seen around both ZrO_2 and *Ti* abutments at all times, without significant differences in most clinical parameters of peri-implant soft tissue health. Mean probing depths around *Ti* abutments were slightly deeper than around ZrO_2 abutments after 3 months (2.2 s.d. 0.8 mm vs 1.7 s.d. 0.7 mm, p=0.03).

Conclusions: No difference in health of the soft tissues adjacent to ZrO_2 and *Ti* abutment surfaces nor in early bacterial colonization could be demonstrated, although somewhat shallower probing depths were observed around ZrO_2 abutments after 3 month.

Introduction

Titanium (*Ti*) has been the 'gold standard' material for implant abutments, but the use of high-strength ceramics, both as permucosal abutments on implants and as copings for ceramic crowns, is increasing. Zirconia (ZrO_2) is especially promising because of its high fracture toughness and favourable light dynamics. To date there is only limited information available with respect to the clinical and biological performance of ZrO_2 -based restorations (Jung et al, 2008b; Zembic et al, 2009; Sailer et al, 2009a). Attention in the literature has predominantly been focused on the bone-implant response to *Ti* and ZrO_2 and on the biomechanical properties of these materials (Wenz et al, 2008). Much less information is available regarding the soft tissue response to ZrO_2 and comparative in vivo studies in humans are quite scarce (Myshin and Wiens, 2005; Teughels et al, 2006; Linkevicius and Apse, 2008).

The establishment and maintenance of healthy soft tissues around implant abutments are considered to be important for long term service of the implant (Lindquist et al, 1996). The intimate contact between the marginal mucosa and implant abutment protects the implant body from the microbial communities of the mouth. As on teeth, periodontal pathogens on implants induce soft tissue infection. It is presumed that this may jeopardize the osseointegration process (Norowski, Jr. and Bumgardner, 2009).

The adhesion, proliferation and colonization of cells and micro-organisms is dependent upon the surface properties, among which are its biocompatibility (i.c. chemistry), surface topography (i.c. roughness) and surface-free energy (Quirynen et al, 1993; Quirynen et al, 1994; Bollen et al, 1996; Rimondini et al, 1997; Abrahamsson et al, 1998; Rasperini 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 and Apse, 2008). Bacterial colonization of the abutment starts directly after exposure to the oral environment and within weeks, the subgingival microbiota is similar to that found around teeth in the same mouth (van Winkelhoff et al, 2000; Quirynen et al, 2005; Quirynen et al, 2006; Furst et al, 2007; DeAngelo et al, 2007; Salvi et al, 2008).

Strategies aimed at reducing bacterial adhesion and biofilm formation on implant abutment surfaces are of pertinent clinical interest and can be employed for the maintenance of soft tissue health or possibly in the treatment of peri-implantitis. Recent studies have shown that antimicrobial (e.g. vancomycin or chitosan) derivatization of a *Ti* alloy surface renders it less susceptible for bacterial colonization in vitro (Parvizi et al, 2004; Antoci Jr. et al, 2008; Shi et al, 2008). Implant coatings that deliver antibiotics have been described as well, predominantly in the field of orthopaedics (Norowski, Jr. and Bumgardner, 2009). It was shown that the physical properties of the *Ti* surface can be adapted, for example by applying a coating of *Ti* -nitride through vapour deposition. This reduces plaque adhesion compared to uncoated *Ti* surfaces both in vitro and in vivo (Grossner-Schreiber et al, 2001; Scarano et al, 2003) and still facilitates cellular adhesion of human fibroblasts in vitro (Grossner-

Schreiber et al, 2006). In addition, it has been observed that silver and zinc oxide modified surfaces possess antibacterial properties as well (Norowski, Jr. and Bumgardner, 2009).
Wennerberg et al compared the inflammatory response in human peri-implant mucosa around standard *Ti* abutments and abutments that were roughened by grid blasting. They found no correlation between the number of inflammatory cells and degree of roughness after 4 weeks (Wennerberg et al, 2003). However, creating much smoother surfaces than those generally encountered on currently used *Ti* abutments (Ra–value approx. 35 nm) reduces bacterial adhesion in vitro (Pier-Francesco et al, 2006). Other authors compared *Ti* -abutments with different roughnesses and a smooth ceramic abutment (of undisclosed chemical composition, Ra-value 60 nm). Since fibroblasts require a certain roughness to be able to adhere to a *Ti* substrate, the authors suggest an optimal surface roughness Ra-value of 200 nm. Such roughness constitutes a good balance. It reduces plaque adhesion as compared to a rougher surface yet is still rough enough for fibroblast adhesion and the establishment of a durable epithelial soft tissue seal (Bollen et al, 1996; Quirynen et al, 1996). Interestingly, no difference in early biofilm formation on subgingival abutment surfaces with varying roughnesses could be demonstrated by others (Elter et al, 2008).
The potential advantages of ZrO_2 compared to *Ti* with respect to biofilm formation in the oral cavity has been demonstrated in various studies. ZrO_2 disks that were glued on a device and worn intra-orally for a day elicited less plaque accumulation than *Ti* disks in vivo (Scarano et al, 2004). This finding was attributed to the superficial structure of the ZrO_2 , more specifically, to its electric conductivity. Others reported similar favourable findings in vitro and in vivo in a comparable experiment (Rimondini et al, 2002). These observations were not verified on functional, permucosal abutments. Degidi et al performed a study in 5 patients comparing ZrO_2 and *Ti* in permucosal applications. Less pronounced inflammation-related processes were noticed around ZrO_2 versus *Ti* healing abutments after 6 months (Degidi et al, 2006). The peri-implant microbiota was not investigated in the latter study.
The present investigation focuses on the peri-implant mucosa condition adjacent to ZrO_2 and *Ti* abutment surfaces and on early submucosal bacterial colonization. These issues are compared under the null hypotheses that permucosal sites adjacent to ZrO_2 and *Ti* abutment surfaces exhibit similar clinical characteristics of peri-implant soft tissue health and microbiological features during the first 3 months.

Materials and methods

The study was designed as a prospective, human, within-subject comparison with left-right randomisation. Twenty edentulous patients, 9 males and 11 females, aged between 39 and 76 years (mean, 56.4 years) who were scheduled for 2 mandibular implants and overdenture treatment, were enrolled in the study. Inclusion criteria were:

- reasonable to good general health, as expressed by a score I or II on the physical status classification system by the American Association of Anesthesiologists (ASA-score);

- bone height in the mandibular anterior region allowing the placement of 11, 13 or 15 mm screw implants. Bone width had to be such that implants of 3.5 or 4.0 mm in diameter could be placed;
- no history of previous implant loss, no pathology or irradiation of the (anterior) mandible. The study protocol was approved by the medical ethics committee of the University Medical Center Utrecht and written informed consent was obtained.

Implant installation

Two *Ti* screw implants (Astra Tech Implant System, OsseoSpeed™ implants, Dentsply Implants, Mölndal, Sweden) were placed in 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 100mg) and rinsed with a 0.2% chlorhexidine solution from 2 days preoperatively until 2 weeks postoperatively.
Implant diameter and length within each subject were similar. The implants were placed and randomised to immediately be provided with either one (experimental) ZrO_2 or one *Ti* abutment, functioning as a permucosal healing abutment.
Two weeks after surgery brushing was allowed. Subjects were enrolled in a strict follow-up protocol that focused on oral hygiene, but during the experimental period, the abutments were never professionally cleaned.

Abutments (ZrO_2 and *Ti*)

The experimental abutments were especially designed, fabricated and CE-marked for the study and are not commercially available. Bulk material for the *Ti* abutments was *Ti*, grade 4, according to ASTM F-67 and Y-TZP according to ISO 13356 for the ZrO_2 specimen (Astra Tech Implant System, Dentsply Implants, Mölndal, Sweden). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular *Ti* and ZrO_2 abutments by the same manufacturer (e.g. the Astra Tech Implant ZirDesign™ and TiDesign™ abutments, Dentsply implants, Mölndal, Sweden). Surface finish requirements for both abutment types were also similar to ordinary production.
The surface roughness of the experimental ZrO_2 and *Ti* abutments was measured at 3 locations on one specimen of each material by means of contact profilometry. Mean R_a-values were 236 nm (range: 217-255 nm) for the ZrO_2 abutment and 210 nm (range: 173-272 nm) for the *Ti* abutment. The corresponding Rq-values were 292 nm (range 260-330 nm) and 259 nm (range 220-332 nm) for the ZrO_2 and *Ti* abutments. Hence, the surface roughness of the materials used was considered to be in the same order of magnitude, and the main difference between the 2 experimental abutments is their chemical composition.
The location for the ZrO_2 and *Ti* abutment (left/right) were allotted at random in such a way that the distribution over the 20 patients resulted in a balanced design.

Microbiological sampling and follow-up

Microbiological sampling and measurement of clinical parameters were performed at 2 weeks and 3 months postoperatively. Sulcular plaque samples were obtained by performing a circumferential motion (360 degrees) in the peri-implant sulci with a sterilized single use plastic scaler (Implacare®, Hu-Friedy, Rockwell st, United States).

Microbiological analysis

Detection and counting of the numbers of *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), Parvimonas micra (Pm), Fusobacterium nucleatum (Fn) and Treponema denticola (Td)* were performed using Real-Time PCR as described by others (Kuboniwa et al, 2004; Boutaga et al, 2005). In brief, amplification of species-specific 16S rDNA sequences was performed in a 20-µL reaction mixture containing 10 µL of 2x LightCycler® 480 Probes Master (Roche), 300 nM of species-specific primers, 100 nM of a species-specific probe (both from TIB MolBiol GmbH, Berlin, Germany; modified by a FAM reporter and a BHQ-2 quencher), and 5 µL of DNA purified from the plaque samples. The sequences of species-specific primers and probes have been described by Boutaga and co-workers (Boutaga et al, 2003; Boutaga et al, 2005) and those for T. denticola by Kuboniwa and co-workers (Kuboniwa et al, 2004). Five microliters of the DNA extracted from the following well-defined reference strains was used to prepare a standard curve as positive controls: P. gingivalis strain HG66 (W83), T. forsythia ATCC 43037, A. actinomycetemcomitans NCTC 9710, P. intermedia ATCC 25611, F. nucleatum ATCC 25586, P. micra HG 1179 (ATCC 33270), and T. denticola (ATCC 33520); 5 µL of sterile H2O was used as a non-template control.

The samples were subjected to an initial single incubation at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s and 60°C for 20 s. DNA amplification was monitored by quantitatively analyzing the fluorescence emission (LightCycler 480, software version 1.5, Roche) during each annealing-extension step.

Clinical parameters

Probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were assessed at 2 sites per implant (mid-buccal and mesial). A plastic periodontal probe with 0.25 N of calibrated probing force was used (Click-probe®, KerrHawe, Bioggio, Switzerland). PPD was measured in millimetres from the mucosal margin to the clinical pocket. REC was measured in millimetres from the edge of the abutment to the mucosal margin (Figure 1). BOP was recorded as absent (score=0) or present (score=1). Mean values per implant were calculated for the continuous parameters (meanPPD, meanREC). BOP is presented as the percentage of implants that demonstrated either mid-buccal or mesial bleeding on probing.

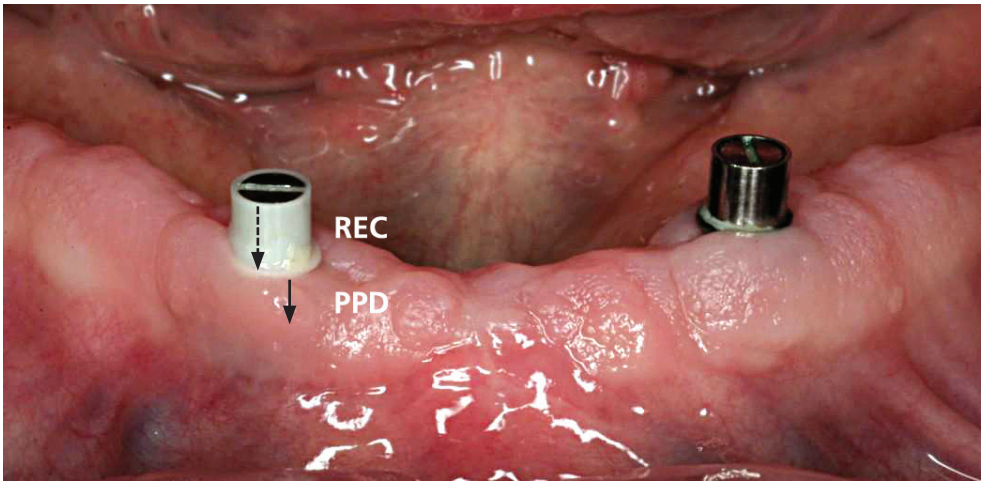

Figure 1. Zirconia and titanium experimental abutments in situ after 3 months. Recession is measured from the edge of the implant to the mucosal margin (REC). The pocket probing depth is measured from the mucosal margin to the clinical pocket (PPD).

Statistical analysis

The mean values for the clinical parameters and levels of the 7 marker bacteria associated with the ZrO_2 and *Ti* abutments were described and statistically compared at 2 weeks and after 3 months post-surgery. Non-parametric statistical procedures were used for all comparisons (Wilcoxon matched-pairs, sign-rank test). All statistical computations were performed in a standard statistical program (SPSS version 16, SPSS Inc, Chicago, United States). Statistical significance of the comparison between the ZrO_2 and *Ti* abutments and the 2 observation periods was set at $p<0.05$.

Results

Data at 3 months in one subject could not be recorded because of a breach of protocol. The experimental abutments had already been removed prior to microbiological sampling and clinical measurement taking.

Mean values for the clinical parameters of the peri-implant mucosa surrounding the ZrO_2 and *Ti* abutments at 2 weeks and at 3 months are presented in table 1 (meanPPD, meanREC, BOP). Mean probing depths at 3 month were shallower around ZrO_2 compared to *Ti* abutments. No further statistically significant clinical differences between ZrO_2 and *Ti* abutments were observed for meanPPD, meanREC or BOP at 2 weeks or 3 months. The meanPPD's decreased significantly for both the ZrO_2 and the *Ti* abutments between 2 weeks and 3 months. In contrast, meanREC increased in time for both abutment types. Slightly less bleeding on probing was observed around the *Ti* abutments at 3 months compared to the observations 2 weeks post operatively (Table 1).

Table 1. Evaluation of mean pocket probing depth (meanPPD), mean recession (meanREC) and bleeding on probing (BOP, either buccal or mesial). Pairwise comparison of data after 2 weeks and 3 months for zirconia (ZrO_2) and for titanium (*Ti*) abutments (Wilcoxon matched-pairs test, sign-rank test). Standard deviations between brackets (n=20 subjects for the 2 weeks and 19 subjects for the 3 month interval)

		2 weeks	3 months	p-level
meanPPD	ZrO_2	3.0 (1.1)	1.7 (0.7)	$Z_{-3.65}$, *p=0.00*
	Ti	2.9 (0.8)	2.2 (0.8)	$Z_{-3.01}$, *p=0.00*
		$Z_{0.14}$, *p=0.89*	$Z_{-2.16}$, *p=0.03*	
meanREC	ZrO_2	2.1 (1.2)	2.7 (0.6)	$Z_{-2.49}$, *p=0.01*
	Ti	1.9 (1.2)	2.6 (1.0)	$Z_{-2.82}$, *p=0.00*
		$Z_{-0.97}$, *p=0.14*	$Z_{-0.32}$, *p=0.98*	
BOP	ZrO_2	50.0%	52.6%	$Z_{-0.25}$, *p=0.80*
	Ti	75.0%	47.4%	$Z_{-2.01}$, *p=0.05*
		$Z_{-0.83}$, *p=0.41*	$Z_{-1.19}$, *p=0.23*	

Table 2. The number of peri-implant sites with detectable levels of 7 periodontal bacterial species using RT PCR, 2 weeks and 3 months after installation of the zirconia (ZrO_2) and titanium (*Ti*) abutments (n=20 subjects for the 2 weeks and n=19 subjects for the 3 month interval). Mean absolute counts (mean) for those observations exceeding the detection threshold and their standard deviation (sd) as well as the median values are presented.

	Aa		Pg		Pi	
2 weeks (n=20)	ZrO_2	*Ti*	ZrO_2	*Ti*	ZrO_2	*Ti*
N detected	1	2	0	0	3	1
ZrO_2+/*Ti*-	1		0		3	
ZrO_2-/*Ti*+	2		0		1	
ZrO_2=*Ti*	17		20		16	
Mean	4620	220	0	0	4150	91
sd	0	199	0	0	6802	0
Median	4620	220	0	0	440	91

	Aa		Pg		Pi	
3 months (n=19)	ZrO_2	*Ti*	ZrO_2	*Ti*	ZrO_2	*Ti*
N detected	0	0	1	2	4	5
ZrO_2+/*Ti*-	0		0		0	
ZrO_2-/*Ti*+	0		1		1	
ZrO_2=*Ti*	19		18		18	
Mean	0	0	1000000	64000	600088	3600089*
sd	0	0	0	36770	952117	804935
Median	0	0	1000000	64000	200090	42

* statistically significant difference between 2 weeks and 3 months, p<0.05.

The numbers of peri-implant sites with detectable levels of 7 periodontal bacteria at 2 weeks and at 3 months are presented in table 2. The cumulative bacterial load is described per subject in table 3. No statistically significant difference could be observed in counts of the 7 marker bacteria or in cumulative bacterial load between the *ZrO_2* and *Ti* abutments, both at 2 weeks and at 3 months. Generally, slightly larger numbers of bacteria were found at the *ZrO_2* abutment surfaces compared to the *Ti* surfaces, although this never reached a statistically significant level (Table 2).

At two weeks, the most frequently detected periodontal species were *P. micra* and *F. nucleatum*. In contrast, periodontal pathogens such as *A. actinomycetemcomitans, P. gingivalis* and *T. denticola* were not detectable in the majority of patients (Table 2). *A. actinomycetemcomitans* was detected in 3 subjects at 2 weeks post surgery, but was no longer detectable at 3 months in any of test sites. Two subjects hosted *P. gingivalis* at 3 months, but not at 2 weeks.

The bacterial colonization of *ZrO_2* surfaces did not undergo major changes between 2 weeks and 3 months, although a slight increase of *F. nucleatum* cells was observed ($Z_{-1.97}$, p=0.05). At *Ti* abutment surfaces, the counts of *P. intermedia* ($Z_{-2.02}$, p<0.05) and *P. micra* ($Z_{-2.10}$, p<0.05) increased statistically significant in time (Table 2).

Tf		Pm		Fn		Td	
ZrO_2	*Ti*	*ZrO_2*	*Ti*	*ZrO_2*	*Ti*	*ZrO_2*	*Ti*
1	0	7	6	17	15	2	1
1		4		2		2	
0		3		1		1	
19		13		17		17	
22000000	0	45395	16184	126465	13467002	49503	280
0	0	94798	33530	434701	4810665	69999	0
22000000	0	4100	400	1540	1070	49504	280

Tf		Pm		Fn		Td	
ZrO_2	*Ti*	*ZrO_2*	*Ti*	*ZrO_2*	*Ti*	*ZrO_2*	*Ti*
1	0	11	11	17	17	2	0
0		2		0		2	
0		2		0		0	
19		15		19		17	
3700	0	221651	170351*	1728753	702662	280	0
0	0	338980	509342	4555954	1548894	170	0
3700	0	3800	2000	120000	37000	280	0

Table 3. Cumulative bacterial load of 7 periodontal bacterial species using RT PCR on zirconia and titanium abutment surfaces at 2 weeks and 3 months post surgery (n=20 subjects for the 2 weeks and 19 subjects for the 3 month interval). Data are presented per subject, in absolute counts per sample and pairwise compared (Wilcoxon matched-pairs, sign-rank test). Sites where the detection threshold was not exceeded are awarded the value '0'.

	2 weeks		3 months		ZrO_2		Ti	
Subject	*ZrO_2*	*Ti*	*ZrO_2*	*Ti*	2 weeks	3 months	2 weeks	3 months
1	0	0	-	-	0	-	0	-
2	1700	69000	1810000	106000	1700	1810000	69000	106000
3	298631	19390200	853000	1433000	298631	853000	19390200	1433000
4	1540	1249	1280000	2830	1540	1280000	1249	2830
5	114	4260	400019	2260000	114	400019	4260	2260000
6	3006	266	690620	2000862	3006	690620	266	2000862
7	220000	220000	2300	2500	220000	2300	220000	2500
8	50240	0	0	0	50240	0	0	0
9	30410	170	3300	220	30410	3300	170	220
10	1804110	1420	124200	112200	1804110	124200	1420	112200
11	22213000	66000	4900	2175	22213000	4900	66000	2175
12	3230	290	3400	3400	3230	3400	290	3400
13	3100	1540	650170	3830	3100	650170	1540	3830
14	650	650	4800160	9500000	650	4800160	650	9500000
15	340	400	16000	1200	340	16000	400	1200
16	0	374	4530000	110540	0	4530000	374	110540
17	656	970	48000	32400	656	48000	970	32400
18	43000	4500	3200	1500	43000	3200	4500	1500
19	58	0	8600	38900	58	8600	0	38900
20	750	1900041	2903700	136000	750	2903700	1900041	136000
Summary	*Ti>ZrO_2: 7*		*Ti>ZrO_2: 6*		*$ZrO_2$2w>$ZrO_2$3m: 6*		*Ti2w>Ti3m: 5*	
statistic	*Ti<ZrO_2: 10*		*Ti<ZrO_2: 11*		*$ZrO_2$2w<$ZrO_2$3m: 13*		*Ti2w<Ti3m: 13*	
	Ti=ZrO_2: 3		*Ti=ZrO_2: 2*		*$ZrO_2$2w=$ZrO_2$3m: 0*		*Ti2w=Ti3m: 1*	
	$Z_{-0.59}$, p=0.55		*$Z_{-0.73}$, p=0.46*		*$Z_{-1.53}$, p=0.13*		*$Z_{-1.11}$, p=0.27*	

Discussion

Zirconia is becoming a favoured material in restorative dentistry for implant abutments and as copings for crowns and bridges, mainly because of its presumed favourable light dynamics. In a way, this is somewhat worrying considering the fact that long term clinical data documenting the performance of *ZrO_2* abutments and restorations are scarce. The same can be said with respect to the soft tissue response to *ZrO_2* itself, since well controlled in vivo human studies are lacking as was also postulated in a consensus statement on soft tissue integration (Klinge and Meyle, 2006). The present study deals with the peri-implant soft tissue response to *ZrO_2* and *Ti* implant abutments and the early bacterial colonization.

The choice for a within-subject comparison in edentulous subjects was made because it offered the best possibility for eliminating confounding factors. For example,the bacterial challenge by the oral microflora is the same in one individual. As a result, implant dimensions and many other variables within the same subject were similar in all cases and microbiological sampling and clinical procedures could be standardised as much as possible. Since the surface roughness of the experimental abutments made from ZrO_2 and *Ti* was also more or less similar, potential differences in soft tissue response and in bacterial colonization are presumably the result of differences in the chemical composition and consequently of differences in surface-free energy (electrical conductivity). The surface roughness of the abutments that were used (R_a-values 210-236 nm) approached the optimal roughness that was suggested in the literature for permucosal implant abutments (Bollen et al, 1996; Quirynen et al, 2006).

Only a few reports describe longitudinal changes of the subgingival microflora after changing substrata or during implantation (Lee et al, 1999; Furst et al, 2007). In the present study, a detection-method was chosen comprising a high specificity and sensitivity towards pathogenic bacterial species that are related to peri-implant infection. Therefore, we have not performed an investigation method that gives an overview of 'all species', like anaerobic culture or a - money-wise expensive - molecular technique as next-generation sequencing, although the latter would be a very promising option (Zaura et al, 2009). Another advantage of the chosen method was that it enabled us to really quantify the numbers of bacterial cells per species during the evaluation period. Other techniques as DNA-DNA checkerboard hybridization are semi-quantitative only. A real-time PCR is more precise in that way. However, it should be mentioned that a closed target method as real-time PCR might result in an underestimation of changes in bacterial colonization on both ZrO_2 and *Ti* surfaces.

In general, comparable micro-organisms are found around newly placed implants and the remaining dentition. This can also include periodontopathogens as *P. gingivalis* and *A. actinomycetemcomitans*, which might even be a risk for future peri-implant infections (Leonhardt et al, 1999). However, it was to be expected that colonization of implants by such pathogens is restricted to partially edentulous patients, because of the remaining presence of a specific niche, e.g. the periodontal sulcus (Van Winkelhoff et al., 2000). However, recent findings by Assche and coworkers using real time PCR techniques to determine the presence of periodontopathogens reveal that such bacteria will remain at mucosal sites after full-mouth extraction (Van Assche et al, 2009). This might explain our observation of the presence of A. actinomycetemcomitans at implant sites in three edentulous patients and P. gingivalis in two patients.

As in the majority of clinical studies dealing with the evaluation of dental implants and soft tissue health, the condition of the peri-implant mucosa was monitored by means of probing pocket depths, recession and the assessment of a bleeding index. The use of such 'periodontal' parameters to determine the clinical condition of the soft peri-implant tissues has been subject to debate (Ow et al, 1999; Verhoeven et al, 2000). These parameters were used because of the lack of reliable, more sensitive, clinical measures to assess the biological

response of peri-implant mucosa. The effect of the antibiotics used peri-operatively will presumably have affected the soft tissue response at the 2 weeks measurements and not so much so after 3 months. This will be the case for both abutments in a similar manner because of the split mouth study design.

No significant difference in the mean values for probing pocket depth (PPD) were observed between ZrO_2 and *Ti* abutments at 2 weeks. However, at 3 months the permucosal seal around the ZrO_2 abutments seemed somewhat less sensitive to probe penetration as compared to that around the *Ti* abutments (p=0.03). Because the 2 time points were analyzed separately, the chance on false positive findings has increased and statistically significant observations with p-values in the vicinity of 0.05 should be interpreted with caution. In addition, it should be noted that the geometry of the abutments used (Figure 1) may have played a role and hampered reliable probe penetration. A comparison with probing depth measurements, as obtained in other studies does not seem appropriate. The mean values for recession (REC) and bleeding on probing (BOP) were more or less similar at all times for both materials. With respect to the latter it is interesting to note that in a clinical study on the performance of ZrO_2 and *Ti* abutments after 1 and 3 years of function, slightly more bleeding on probing occurred around the ZrO_2 abutments as compared to the *Ti* abutments (Zembic et al, 2009; Sailer et al, 2009a). This was not apparent in the present investigation. It has been suggested that the use of 0.25 N of calibrated probing force (Click-probe®, KerrHawe, Bioggio, Switzerland) induces epithelial bleeding in the absence of soft tissue infection (false positive observations) (Gerber et al, 2009).

Between 2 weeks and 3 months after implant installation, the permucosal tissues undergo some changes. Probing depths decrease and the amount of recession increases, irrespective of the abutment type.

There was no significant difference between the ZrO_2 and *Ti* abutments in the prevalence nor in the counts of any of the 7 marker bacteria, both at 2 weeks and at 3 months (Tables 2 and 3). Hence, a marked qualitative or quantitative difference in the early bacterial colonization of ZrO_2 and *Ti* abutment surfaces was not observed. In in vitro and in vivo studies where the colonization of bacteria was investigated on intra-orally worn ZrO_2 and *Ti* disks, that were embedded in removable prosthetic appliances, ZrO_2 disks harboured less bacteria (Rimondini et al, 2002; Scarano et al, 2004). Such a difference was not found in the present study which may be explained by the different techniques of sampling. It has been suggested that the use of intraoral disks is confounded by tongue and cheek activity (Heuer et al, 2007; Elter et al, 2008).

In a study on early biofilm formation on implant abutments, *A. actinomycetemcomitans* and *P. gingivalis* were not detected on any of 14 titanium healing abutments in 10 patients after 12 days. The authors did not disclose whether the subjects were partly or fully edentulous (Heuer et al, 2007). In the present study *A. actinomycetemcomitans* and *P. gingivalis* were infrequently detected in a very small number of patients.

Overall, on the basis of the studied biological and microbial parameters there are no compelling grounds to favour one abutment material over the other after 3 months.

Considering the limitations that are associated with the use of the rather robust parameters of soft tissue health that were employed (PPD, REC and BOP), the histological data that are currently being evaluated might reveal more subtle differences in soft tissue response towards ZrO_2 and *Ti* abutment surfaces.
Overall, the null hypotheses that permucosal sites adjacent to ZrO_2 and *Ti* abutment surfaces exhibit more or less similar clinical characteristics of peri-implant health and microbiological features during the first 3 months could not be convincingly rejected for most parameters with the exception of the pocket probing depth. Somewhat shallower probing depths were observed around ZrO_2 abutments after 3 months.

Acknowledgement

The authors are grateful for the help of mr. Martijn Martens and dr. Joop Wolke of the Radboud Medical Centre Nijmegen, department of biomaterials and periodontology for determining the surface roughness of the abutments used in this study. Dr. Jan Ruijter of the University Medical Center of Amsterdam, department of anatomy and embryology is recognised for providing statistical advise.

Chapter 3

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

R van Brakel
GJ Meijer
JW Verhoeven
J Jansen
C de Putter
MS Cune

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Abstract

Aim: To compare the response and health of the soft tissues towards Zirconia and Titanium implant abutments in man, as observed histologically.

Materials and Methods: Twenty edentulous patients received 2 mandibular implants that were provided with either a Zirconia or Titanium abutment during implant installation (split mouth study design, left-right randomisation). After 3 months of function soft tissue biopsies were obtained and prepared for histological evaluation. The specimen were subjected to blind evaluation per patient, both qualitatively and quantitatively. The number of blood vessels per surface unit was the primary outcome variable.

Results: Paired samples from 17 patients were deemed suitable for further analysis after histological preparation, 3 with unsevered implant-abutment connections and 14 containing solely soft tissue. All specimen 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. No difference in vascular density in selected regions of interest was observed.

Conclusion: No distinct differences with respect to soft tissue health were seen in peri-implant mucosa adjacent to Zirconia and Titanium abutment surfaces. They appear to elicit a similar soft tissue response in man.

Introduction

Titanium (*Ti*) has been the biomaterial of choice for dental implants for decades because of its favourable, longlasting and well established response under functional loading towards both the hard and soft tissues in the oral environment. These assets can be contributed to its biocompatibility, mechanical strength and corrosion resistance.

However, *Ti* also has potential drawbacks. Increasing demands regarding the esthetics of implant borne restorations have justified the search for other materials with better optical properties. Furthermore *Ti* has relatively little resistance against wear and fretting (Taira et al, 2009). Submicron *Ti* particles may induce inflammatory cytokines secretion in vitro. Attention has also been raised in recent literature regarding potential hypersensitivity towards *Ti* which seems to occur in a limited number of patients and may be caused by the former (Sicilia et al, 2008; Siddiqi et al, 2011; Javed et al, 2013).

High-strength ceramics such as alumina and zirconia (ZrO_2) are currently widely used as permucosal implant abutments and as copings for all-ceramic crowns. Especially ZrO_2 is promising as it allows a CAD-CAM production process, has a high fracture toughness and favourable light dynamics. White ZrO_2 and grayish *Ti* abutments covered by peri-implant mucosa enhance a different light reflection, that is noticeable to the human eye when the covering mucosa is less than 2 mm thick, which is a frequent finding in the clinical situation (Jung et al, 2007; van Brakel et al, 2011b). 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 and Wiens, 2005; Teughels et al, 2006; Klinge and Meyle, 2006; Linkevicius and Apse, 2008; Jung et al, 2008b; Zembic et al, 2009; Sailer et al, 2009a; Nakamura et al, 2010). Bone-implant response to *Ti* and ZrO_2 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 permucosal seal that protects the underlying tissues from the intraoral environment depends heavily upon the adhesion, proliferation and colonization of fibroblastic cells and micro-organisms. Abutment surface properties, among which are biocompatibility (i.e. chemistry), surface topography (i.e. roughness) and surface-free energy are key influencing factors (Quirynen et al, 1993; Quirynen et al, 1994; Bollen et al, 1996; Rimondini et al, 1997; Abrahamsson et al, 1998; Rasperini 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 and Apse, 2008).

Biofilm formation in relation to different substrates is relatively easy to study in humans because it does not require an invasive procedure. ZrO_2 disks on a prosthetic device that were worn intra-orally for a day elicited less plaque accumulation than *Ti* disks. This finding was attributed to the superficial structure of the ZrO_2, more specifically, to its electric conductivity (Rimondini et al, 2002; Scarano et al, 2004; Nakamura et al, 2010; Salihoglu et al, 2011). However, from 2 recent clinical trials involving functional, permucosal ZrO_2 and

Ti abutments no difference in bacterial colonisation was observed. Short-term differences in clinical parameters regarding the soft tissues adjacent to ZrO_2 and *Ti* abutments were also not apparent in these 2 studies, involving both partially and fully edentulous subjects (Salihoglu et al, 2011; van Brakel et al, 2011a).
The present study focuses on the response and health of the soft tissues towards ZrO_2 and *Ti* implant abutments in man as observed histologically. 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 ZrO_2 and *Ti* implant abutments exhibit similar counts of blood vessels after 3 months of intraoral function.

Materials and methods

Twenty edentulous patients, 9 males and 11 females, aged between 39 and 76 years (mean, 56.4 years) who were scheduled for 2 mandibular implants and overdenture treatment were enrolled in the study. Inclusion criteria consisted of:

- reasonable to good general health, as expressed by an ASA-score I or II;
- bone height in the mandibular anterior region allowing the placement of 11, 13 or 15mm screw implants. Bone width should be such that implants of 3.5 or 4.0mm 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 *Ti* screw implants (Astra Tech Implant System, OsseoSpeed™ implants, Dentsply Implants, Mölndal, Sweden) were placed in local anaesthesia in the region of the former mandibular cuspids. Subjects received antibiotics (Vibramycin, from 1 day pre-operatively 200mg until 7 days postoperatively, once daily 100mg) and rinsed with a 0.2% chlorhexidine solution from 2 days preoperatively until 2 weeks postoperatively.
Implant diameter and length of the implants within each subject were similar. They were immediately provided with one (experimental) zirconiumdioxide (ZrO_2) and one *Ti* abutment that functioned as healing abutments (non-submerged implant placement, within-subject comparison, left-right randomisation). 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 (ZrO_2 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 *Ti* grade 4 according to ASTM F-67 and Y-TZP according to ISO 13356 for the ZrO_2 specimen (Astra

Tech Implant System, Dentsply Implants, Mölndal, Sweden, Figures 1 and 2). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular *Ti* and ZrO_2 abutments by the same manufacturer (e.g. the Astra Tech Implant System ZirDesign™ and TiDesign™ abutments, Dentsply Implants, Mölndal, Sweden). Surface finish requirements for both abutment types were also similar. The surface roughness (Ra-value) of the experimental ZrO_2 and *Ti* abutments was measured at 3 locations on one specimen of each material by means of contact profilometry. Mean Ra-values were 236 nm (range: 217-255 nm) for the ZrO_2 abutment and 210 nm (range: 173-272 nm) for the *Ti* abutment. Hence, the surface roughness of the materials used was considered to be in the same order of magnitude and the main difference between the 2 experimental abutments is their chemical composition.

Figure 1. Experimental implant abutment.

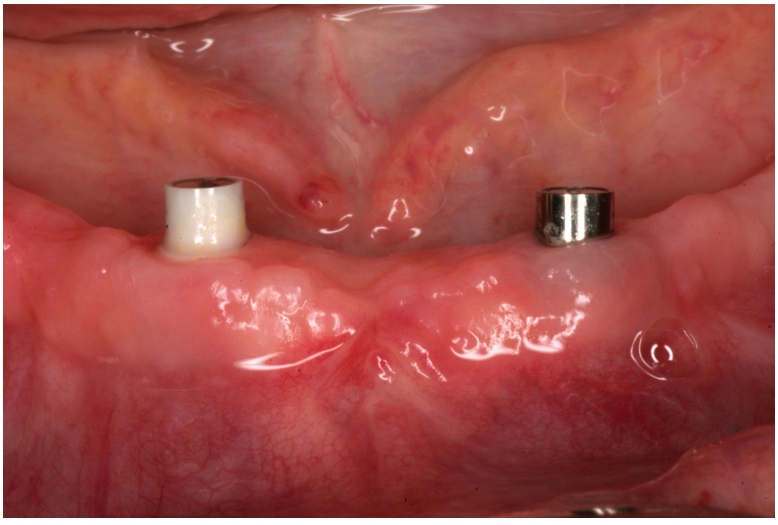

Figure 2. Clinical situation after 3 months with zirconia (left on image) and titanium (right on image) experimental abutments.

Retrieval of biopsies

Biopsies were retrieved 3 months after implant installation, following local anesthesia by injection of Ultracain DS Forte (Sanofi-Aventis, Paris, France). Only in the first three patients it was tried to punch out a biopsy trying to collect the abutment and soft tissue cuff together. Unfortunately, most often only the abutment came out leaving the tissue cuff behind. In addition, patients suffered from serious discomfort, when especially 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 the mesial and distal marginal soft tissue adjacent to the ZrO_2 and *Ti* abutment. These biopsies had the shape of a triangle, 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 until the alveolar bone. A suture was placed at the tip of the triangular biopsy as a reference (Figure 3). During retrieval of the biopsies, the soft tissue and the abutment usually got separated. The 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 hours and subsequently dehydrated in ethanol. The biopsies in which abutments and soft tissues remained undisturbed were embedded in methylmethacrylate (n=3 patients, MMA). Following polymerization, non-decalcified, 10-µm-thick, longitudinal sections of the implants were prepared in a plane perpendicular to the implant using a modified sawing microtome technique and subsequently stained with methylene blue and basic fuchsin (van der Lubbe et al, 1988).

The triangular biopsies containing solely soft tissue 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 hematoxylin-eosin (HE). To elucidate the presence of blood vessels, staining of their vascular basement membrane was performed using collagen IV (Figure 5-6).

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 the quantitative analysis Regions Of Interest (ROI) measuring 200µm by 200µm at the soft tissue – abutment interface were defined for 2 sections per abutment: a mesial and a distal one. The ROI's were positioned at a distance no further than 100µm from the interface and a maximum of 500µm measured from the apical termination of junctional epithelium. The number of blood vessels within each ROI was counted in order to determine vascular density. Mesial and distal sites were averaged.

Statistical analysis and repeatability

The determination of vascular density was repeated for all specimen, in order to asses intraobserver repeatability which is subsequently expressed as the coefficient of repeatability (CR)(Bland and Altman, 1986). The paired samples T-test was used to compare observations adjacent to ZrO_2 and *Ti* abutments in the same patient. All computations were performed in SPSS (SPSS version 17, spss inc, Chicago, United States).

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.

With respect to the unsevered specimen (Figure 3), no difference was observed for the two abutment types. At the abutment site, the collagen fiber bundles were orientated parallel to the abutment surface. The thickness of the epithelium showed normal proportions varying between 0.2-0.5 mm

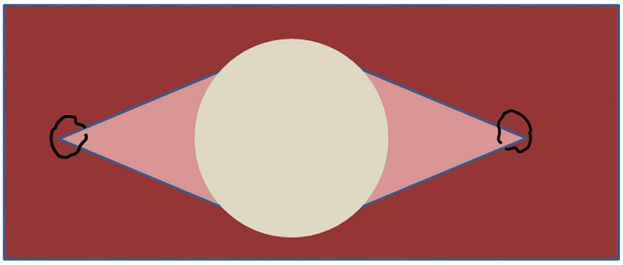

Figure 3. Biopsies obtained mesial and distal from the abutment surface, until bone level. Sutures mark the sites the furthest away from the abutment surface.

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. No differences in qualitative histological features between both groups were noted

Quantitative histology

Based on morphological criteria little inflammation was observed. The CR for intraobserver repeatability with respect to the measurements of vascular density is 8.0. The interpretation of the coefficient of repeatability (CR) was done according to the guidelines of the British Standards, which states that 95% of all measurements should be within two standard

deviations of the mean of the combined 1st and 2nd measurements (British Standards Institution, 1975). This was the case (96 %), hence intraobserver repeatability is considered satisfactory and the duplicate measurements were averaged.
Skewness of the data was reviewed by eyeballing of the normal distribution (histograms), as well as comparison of the median and the mean values. There was no reason to assume that they are skewed. The mean vascular density in tissues adjacent to ZrO_2 and *Ti* implant abutments was 21.2 (s.d. 4.4) and 21.5 (s.d. 3.1) respectively (t=-0.36, p=0.72), hence not statistical significantly different.

Discussion

Zirconia (ZrO_2) is increasingly used in permucosal applications, both as copings for indirect restorations as for implant abutments. When Yttria (Y_2O_3) is added to ZrO_2, favorable 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 reveiled 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 leukocytes, collagen and fibroblasts around abutments made of ZrO_2 and *Ti* in labrador dogs. Both abutments compared favorably 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 biopsies revealed similar dimensions around both implant types (Kohal et al, 2004). In a rare human study in 5 partially edentulous subjects less pronounced inflammation-related processes were noticed around ZrO_2 versus *Ti* healing abutments after 6 months (Degidi et al, 2006).
Although the present study was designed to eventually perform histological evaluation on specimen consisting of abutments and adjacent soft tissues, this proved not feasible in the majority of cases and would have seriously harmed the patients. Nevertheless the obtained histological images of 3 patients showed healthy peri-implant tissues without clear differences in anatomical dimensions between tissue adjacent to the ZrO_2 and *Ti* abutment surfaces.
Blood vessel density, reflecting soft tissue health, between mucosa overlying ZrO_2 and *Ti* abutment surfaces was not statistical significantly different, nor were qualitative

observations. Hence, although plaque seems to favor *Ti* surfaces (Nakamura et al, 2010), this does not seem to bear great clinical relevance and tissues are equally healthy. Thin soft tissues covering *Ti* abutment surfaces appear darker to the human eye and therefore unhealthier than tissues supporting ZrO_2 surfaces (Jung et al, 2007; van Brakel et al, 2011b). More soft tissue color mismatch was seen between natural teeth and implants provided with titanium compared to implants provided with zirconia abutments (Bressan et al, 2011). We presume this is a consequence of blue-grayish shimmering of the *Ti*, not because mucosa reacts less favorably to *Ti* compared to ZrO_2.

It is concluded that no distinct differences with respect to soft tissue health exist in peri-implant mucosa adjacent to ZrO_2 and *Ti* abutment surfaces. They appear to elicit a similar soft tissue response in man. Perceived differences in appearance of soft tissues overlying ZrO_2 and *Ti* abutments may well be optical, rather than biological.

Acknowledgements

The authors are grateful for the help of mr. Martijn Martens, dr. Joop Wolke and Natasja Raaijmakers of the Radboud Medical Centre Nijmegen, department of biomaterials and periodontology for determining the surface roughness of the abutments and for the preparation of the samples. Robin van Rijn, dental student is kindly acknowledged for his help with the histomorphometric procedures.

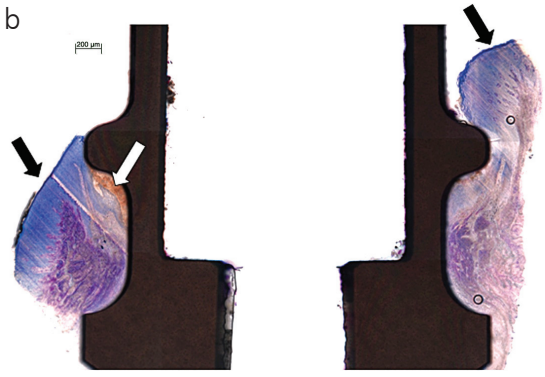

Figure 4a. Overview of a Zirconia abutment including the soft tissue biopsy (unsevered specimen).

Figure 4b. Detail of 4a. Clearly, the transverse stripes (black arrows) are seen. They are induced by the preparation process, thereby blurring the underlying picture. The white arrow points at the brown colored exsudate.

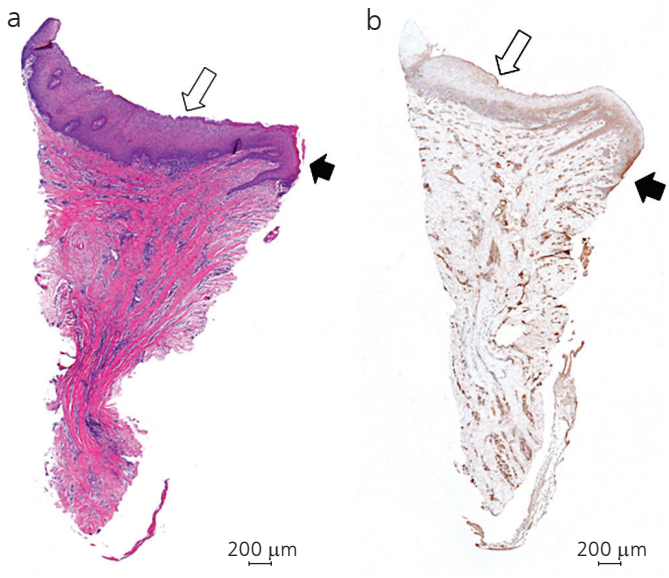

Figure 5a. HE staining showing the intact intraoral mucosa (white arrow) and the barrier epithelium bordering a Titanium abutment (black arrow)

Figure 5b. Collagen IV staining showing the brown borders of the endotheleum cells lining blood vessels.

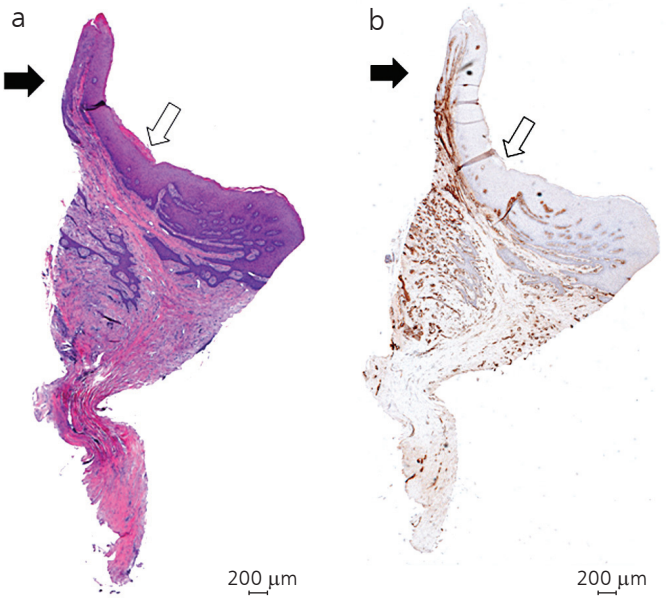

Figure 6a. HE staining, showing the intact intraoral mucosa (white arrow) and the barrier epithelium (black arrow) bordering a Zirconia abutment.

Figure 6b. Collagen IV staining (brown) showing the brown borders of the endotheleum cells lining blood vessels.

Chapter 4

The association of clinical and microbiological parameters with histological observations in relatively healthy peri-implant conditions – A preliminary short–term in vivo study

R van Brakel
GJ Meijer
C de Putter
J Jansen
MS Cune

This chapter is an adapted version of the manuscript: "The association of clinical and microbiological parameters with histological observations in relatively healthy peri-implant conditions – A preliminary short–term in vivo study", that was accepted for publication by the International Journal of Prosthodontics.

Abstract

Aim: To determine whether clinical findings as bleeding on probation, pocketdepth, recession, bacterial sampling and histological findings correlate to statistical outcome of peri-implant soft tissue health in vivo.

Materials and Methods: Twenty edentulous subjects received 2 endosseous mandibular implants. The implants were fitted with either a ZrO_2 or a *Ti* abutment (non-submerged implant placement, within-subject comparison, left-right randomisation). Sulcular bacterial sampling and the assessment of probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were performed 3 months post-surgery. Mucosal biopsies were obtained and the blood vessel density (BVD) and a score on an inflamation grading scale were determined.

Results: Simple linear and linear regression models revealed that the clinical or microbiological parameters are not associated with either of the histological parameters.

Conclusions: Clinical and microbiological parameters are poor predictors of histological parameters in conditions with relatively healthy peri-implant soft tissues *in vivo.*

Introduction

Titanium (*Ti*) has been the gold standard ground implant material over the years. Zirconium dioxide implants (ZrO_2) and abutments are less well documented, with a only a few of studies performed in man, comparing both materials during clinical function. Research focus in general has been primarily focussed on the acceptance of implant materials by bone and the biomechanics of implants and to a lesser degree on the soft tissue response (Wenz et al, 2008).

Tissue response is not only influenced by the chemistry of a material, but also by its surface roughness (Teughels et al, 2006). The bone connecting surface has different demands compared to the peri-implant soft tissue zone. Osseointegration is served well by a certain roughness and coatings, where soft tissue favours relatively smoother surfaces: smooth enough to be unattractive for bacteria, but rough enough for fibroblasts to adhere to. In clinical studies, ZrO_2 and *Ti* abutments do not appear to differ in adhesion and colonization of paropathogens such as A. actinomycetemcomitans and P. gingivalis (Salihoglu et al, 2011; de Oliveira et al, 2012; Nascimento et al, 2014). They seem to elicit a similar soft tissue response (Zembic et al, 2009; Sailer et al, 2009a; Sailer et al, 2009b; van Brakel et al, 2012; de Oliveira et al, 2012). However, pertinent conclusions with respect to the superiority of either material over the other cannot be drawn because of the limited number of studies on the subject (Nakamura et al, 2010).

Non invasive, indirect methods such as probing, sampling of plaque and light reflection can be used to determine the soft tissue health during the healing phase and after abutment connection. When studying tissue response to different abutment materials in humans, methods that more directly reflect tissue health, such as potential differences on a histological level obviously are preferred but rarely found in the literature (Salihoglu et al, 2011).

The present study focuses on the response and health of the soft tissues towards ZrO_2 and *Ti* implant abutments in man as observed both clinically, and histologically. The number of blood vessels per surface unit is the primary outcome variable, as it is presumed to be a direct reflection of soft tissue health. It is hypothesized that soft tissues adjacent to ZrO_2 and *Ti* implant abutments exhibit similar counts of blood vessels after 3 months of intraoral function. Possible associations between clinical, microbiological parameters on the one the hand and histological parameters on the other hand are the primary subject of interest in order to elucidate whether or not they can reliably reflect peri-implant soft tissue health.

Materials 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. 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 and Abraham-Inpijn, 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 and implant abutments

Two *Ti* screw implants (Astra Tech Implant System, OsseoSpeed™ implants, Dentsply Implants, Mölndal, Sweden) were placed under local anesthesia 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 as a result of standard operation procedure at the time.
Implant diameter and length of the implants within each subject were similar as described previously (van Brakel et al, 2011a). They were immediately provided with one (experimental) zirconium dioxide (ZrO_2) and one *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.
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 *Ti* grade 4 according to ASTM F-67 and Y-TZP according to ISO 13356 for the ZrO_2 specimen (Astra Tech Implant System, Dentsply Implants, Mölndal, Sweden, Figure 1). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular *Ti* and ZrO_2 abutments by the same manufacturer (e.g. the Astra Tech Implant System ZirDesign™ and TiDesign™ abutments, Dentsply Implants, Mölndal, Sweden). Surface finish requirements for both abutment types were also similar. The surface roughness of the materials used was in the same order of magnitude and the main difference between the 2 experimental abutments is their chemical composition (van Brakel et al, 2012). Clinical, microbiological and histological data are collected after 3 months of permucosal healing.

Clinical parameters

Probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were assessed at two sites per implant (mid-buccal and mesial). A plastic periodontal probe with 0.25 N of calibrated probing force was used (Click-probe®, KerrHawe, Bioggio, Switzerland). PPD was measured in millimeters from the mucosal margin to the clinical pocket. REC was measured

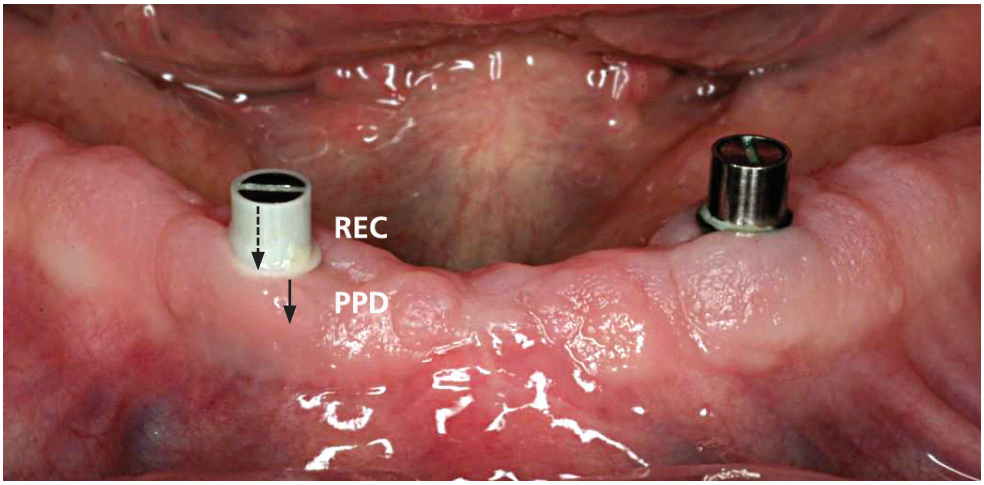

Figure 1. Zirconia and titanium experimental abutments in situ after 3 months. Recession is measured from the edge of the implant to the mucosal margin (REC). The probing depth is measured from the mucosal margin to the clinical pocket (PPD).

in millimeters from the edge of the abutment to the mucosal margin (Figure 1). BOP was recorded as absent (score=0) or present (score=1). Mean values per implant were calculated for the continuous parameters (meanPPD, meanREC). BOP is presented as the percentage of implants that demonstrated either mid-buccal or mesial BOP.

Microbiological parameters: sampling and analysis

Sulcular plaque samples were obtained by performing a circumferential motion (360°) in the peri-implant sulci with a sterilized single-use plastic scaler (Implacare®, Hu-Friedy, Rockwell st, Chicago, IL, USA).

Detection and counting of the numbers of Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), Parvimonas micra (Pm), Fusobacterium nucleatum (Fn) and Treponema denticola (Td) were performed using real-time PCR as described in detail previously (van Brakel et al, 2011a). The total bacterial load is considered as the microbiological outcome measure.

Histological parameters: retrieval of biopsies, preparation and analysis

In the first three patients it was tried to punch out a biopsy in order to collect the abutment and soft tissue cuff together. However, during retrieval of the biopsies, the soft tissue and the abutment usually got separated. In addition, patients suffered from serious discomfort, 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 ZrO_2 and *Ti* abutment in the remaining 17 patients. Tissues from these patients were used in the present study. Consequently, the material comprised of 68 biopsies (17 patients, 2 biopsies, 2 abutments)

that 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. All soft tissue specimens were stored in 4% buffered formaldehyde solution for fixation and transportation to the laboratory prior to histological preparation.

The biopsies 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. To elucidate the presence of blood vessels, staining of their vascular basement membrane was performed using collagen IV. 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 quantitative analysis of the tissue response.

For quantitative analysis Regions Of Interest (ROI) measuring 200 µm by 200 µm 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. Inflammation was also scored on a 4-point scale (Table 1). A higher score represents a better i.c. reduced inflammatory response.

Table 1. Quantitative histological scoring system for inflammation.

Response	Score	Description
Inflammation	1	Masses of inflammatory cells
	2	Many inflammatory cells, showing some fibroblasts
	3	Immature connective tissue, showing fibroblasts with few inflammatory cells
	4	Normal appearance of connective tissue with few inflammatory cells

Statistical methods

Descriptive statistics were used to describe the clinical, microbiological and histological parameters. Pearson's correlation coefficients were calculated. Linear regression models were computed to assess the association between the clinical and microbiological parameters with the dependent variables blood vessel density score and the 4-point inflammation grading score for the ZrO_2 and the *Ti* abutments separately. Hence four linear regression models were produced.

All computations were performed in SPSS (SPSS version 20; SPSS inc., Chicago, IL, USA).

Results

After histological preparation, paired samples from 14 patients containing solely soft tissue were deemed suitable for further analysis. The process for making the histological specimen had failed in three patients for reasons not known. Clinical and microbiological data at 3 months in one subject could not be recorded because of a breach of protocol. The experimental abutments had already been removed prior to microbiological sampling and clinical measurement taking. Consequently, differences between observations around the 2 abutment types and associations between parameters could be calculated in 13 patients (Figure 2).

Mean scores are presented in table 2. No statistically significant differences between abutment types were observed for any parameter.

Pearson's correlation coefficients between all parameters are presented in table 3. The correlations between the clinical and microbiological parameters and the two histological parameters are rather low and not statistically significant, with the exception of the association between recession and pocket probing depth for *Ti* abutments.

The linear regression models explained between 24.9% and 65.5% of the observed variation, and none of the clinical or microbiological parameters were statistical significantly associated with either the blood vessel density or the inflammation grading score.

Discussion

The use of traditional periodontal parameters when evaluating peri-implant soft tissue health and the severity of peri-implant disease is common clinical practice, both during regular checkups as in longitudinal observational studies. These include plaque assessment, mucosal conditions, peri-implant probing depth, width of the peri-implant keratinized mucosa, peri-implant sulcus fluid analysis, suppuration, implant mobility and discomfort, resonance frequency analysis and radiographic evaluation (Salvi and Lang, 2004). The focus of the present study was on the relationship between some of these rather blunt periodontal measures of evaluation versus signs of soft tissue health as seen histologically. The latter is regarded to be a more direct representation of soft tissue health: the gold

Table 2. Mean value and standard deviation (s.d.) for clinical, microbiological and histological parameters (n=13 patients and 26 abutments). Paired samples t-test.

	Zirconia abutment	Titanium abutment	t	p-value
Blood Vessel Density	20.9 (s.d. 4.4)	21.1 (s.d. 3.3)	-0.27	0.79
Histology Grading Scale	3.1 (s.d. 0.6)	3.2 (s.d. 0.6)	-0.62	0.55
Bacterial Load	2.0 x 106 (s.d. 5.5 x 106)	9.2 x 106 (s.d. 26.4 x 106)	0.98	0.35
Mean Pocket Probing Depth	1.8 (s.d. 0.5)	2.1 (s.d. 0.8)	-1.39	0.19
Mean Recession	2.7 (s.d. 0.6)	2.7 (s.d. 1.1)	0.0	1.0
Mean BOP	38.5 (s.d. 36.3%)	62.5 (s.d. 46.3%)	-1.20	0.25

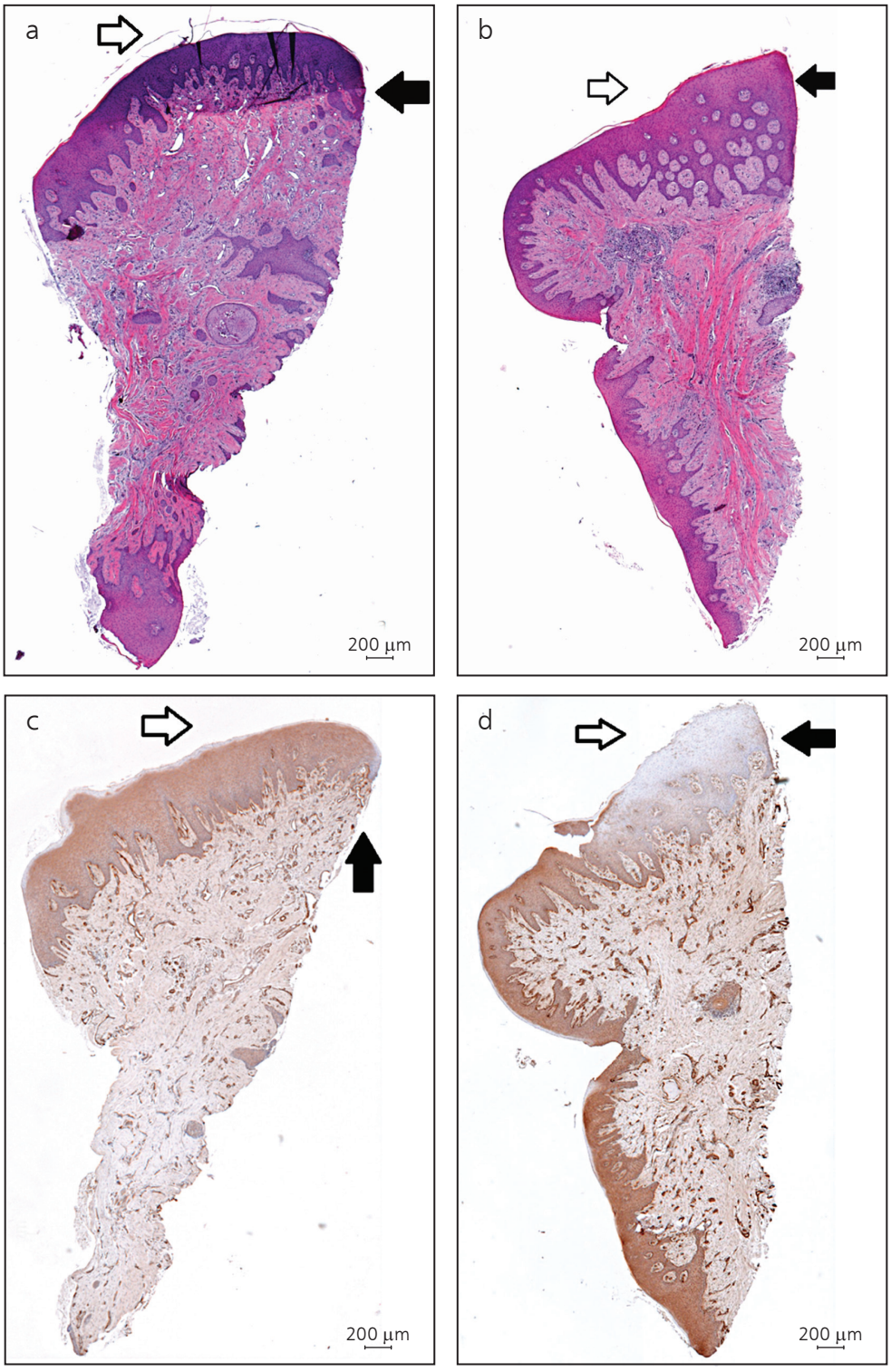
a
200 µm
b
200 µm
c
200 µm
d
200 µm

Figure 2.a HE staining, showing the intact intraoral mucosa (white arrow) and the barrier epithelium (black arrow) bordering the Titanium abutment.

Figure 2.b HE staining, showing the intact intraoral mucosa (white arrow) and the barrier epithelium (black arrow) bordering the Zirconiumdioxide abutment.

Figure 2.c Collagen IV staining (brown), showing the intact intraoral mucosa (white arrow) and the barrier epithelium (black arrow) bordering the titanium abutment.

Figure 2.d Collagen IV staining (brown), showing the intact intraoral mucosa (white arrow) and the barrier epithelium (black arrow) bordering the Zirconiumdioxide abutment.

Figures a to d are representative images from the study and no statistically significant differences between abutment types were observed for any parameter.

standard. Periodontal parameters must be considered indirect indicators. False positive and false negative conclusions based on clinical evaluation would either induce unnecessary care or delay preventive or curative treatment to resolve tissue inflammation. For this purpose, data from a clinical trial comparing soft tissues adjacent to titanium and zirconia implant abutments were used. Data from 13 abutment pairs could eventually be used. Human histological specimen with respect to the soft tissue seal is rarely reported upon and hard to obtain, but the sample size of the present study is consequently rather small.

The experimental implant abutments were designed with retentive grooves, intended to retain biopsied tissues in close proximity to the abutment after removal. This idea was tested prior to the start of the clinical study in an animal cadaver (pig jaw) pilot and seemed feasible. However, in clinical practice the circular biopsy proved to be too invasive for uncompromised healing. Due to long lasting edentulism there is usually little keratinized mucosa preserved in the mandible. For a stable peri-implant situation it is important to keep whatever keratinized mucosa present intact (Lin et al, 2013). Bilateral triangular biopsies were used to obtain the tissue samples.

Biopsied samples are stable after embedding and the preparation of histological samples. Examination is reproducible. Also more than one type of assessment is possible to determine tissue health and inflammatory reaction in the samples. Histological examination consisted of a count of the number of inflammatory cells per surface unit and a qualitative score on inflammation. No differences in both histological parameters between both groups (zirconia versus titanium) were noted. In general, healthy soft tissues were observed when judged histologically.

Clinical evaluation and bacterial sampling was performed by a single physician in an attempt to keep sampling, probing and other measurements as uniform as possible. By nature, they are less reproducible than histological examination. Differences in clinical and microbiological parameters between zirconia and titanium abutments were not observed and tissues appeared generally healthy. These findings are in agreement with other studies (Zembic et al, 2009; Sailer et al, 2009a; Sailer et al, 2009b; de Oliveira et al, 2012; Lops et al, 2013).

The use of a standardized probing pressure (Hawe Neos, Click Probe) guaranteed the least possible inter-observer variability although firmer tissue conditions around the abutments

Table 3. Matrix of Pearson correlations between clinical, microbiological and histological parameters (n=13 patients and 26 abutments).

		HGS	BL	mPPD
Blood Vessel Density (BVD)	ZrO_2	r=-0.14, p=0.65	r=0.38, p=0.20	r=0.10, p=0.74
	Ti	r=0.13, p=0.68	r=-0.19, p=0.54	r=0.06, p=0.86
Histology Grading Scale (HGS)	ZrO_2		r=-0.20, p=0.96	r=-0.19, p=0.53
	Ti		r=0.46, p=0.11	r=0.30, p=0.32
Bacterial Load (BL)	ZrO_2			r=-0.12, p=0.63
	Ti			r=0.24, p=0.33
Mean Pocket Probing Depth (mPPD)	ZrO_2			
	Ti			
Mean Recession (mREC)	ZrO_2			
	Ti			

would make it more difficult to uniformly probe past the retentive groove of the abutments. The shape of the abutment might have been of influence to peri-implant measurements, especially those on bleeding on probing (BOP). The former was seen in 39-62% of the implants in the present study compared to 31% 'peri-implant mucositis' of implants in a recent systematic review (Atieh et al, 2013). The relatively large number of implants that exhibited BOP may have resulted from damaging relatively healthy sulcular epithelium, while probing past the circular groove of the experimental abutment. On the other hand, it may raise questions towards the appropriateness of this and other periodontal parameters to reflect peri-implant soft tissue health, since the histological data revealed healthy soft tissues and the histological and periodontal parameters did not correlate at all. An obvious (but ethically difficult to overcome) shortcoming of this in vivo study is the fact that no pathological conditions were seen nor generated. As a consequence the findings can not be generalized. Hence they may merely demonstrate the (natural) variation in clinical and microbiological parameters under healthy conditions, but underscore the suggestion that periodontal parameters and histological or radiographic evaluation of implant performance are not always in sync either (Verhoeven et al, 2000; Cune et al, 2010).

It is concluded that zirconia and titanium implant abutments elicit a similar soft tissue response when judged clinically, microbiologically and histologically in man. Significant associations between the clinical and microbiological findings on the one hand and histological observations on the other hand were not found. The data give rise to concerns as to the sensitivity and specificity of clinical and microbiological parameters as indicators of the peri-implant soft tissue status in relatively healthy conditions in vivo.

Acknowledgement

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	mREC	mBOP
	r=-0.35, p=0.24	r=0.26, p=0.39
	r=0.01, p=0.96	r=0.06, p=0.84
	r=0.42, p=0.15	r=0.22, p=0.47
	r=-0.32, p=0.29	r=-0.10, p=0.74
	r=-0.01, p=0.98	r=-0.10, p=0.68
	r=0.05. p=0.84	r=-0.36, p=0.14
	r=-0.54, p=0.02**	r=0.10, p=0.66
	r=-0.62, p=0.01**	r=-0.22, p=0.37
		r=0.20, p=0.41
		r=-0.22, p=0.37

Chapter 5

The effect of zirconia and titanium implant abutments on light reflection of the supporting soft tissues

R van Brakel
HJ Noordmans
C de Putter
J Frenken
F Huisman
R de Roode
GC de Wit
MS Cune

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Abstract

Objectives: To determine the difference in light reflection of oral mucosa covering titanium (*Ti*) or zirconia (ZrO_2) abutments as it relates to the thickness of the covering mucosa.

Material and Methods: Fifteen anterior implants (Astra Osseo speed®, Astra Tech Mölndal, Sweden) in 11 patients were fitted with a *Ti* or a ZrO_2 abutment (crossover, within subject comparison). Hyper-spectral images were taken with a camera fitted on a surgical microscope. High resolution images with 70 nm interval between 440 and 720 nm were obtained within 30 seconds (1392x1024 pixels). Black- and white-point reference was used for spatial and spectral normalization as well as correction for motion during exposure. Reflection spectra were extracted from the image on a line mid-buccal of the implant, starting 1 mm above the soft-tissue continuing up to 3 mm's apically.

Results: Median soft-tissue height is 2.3 mm (min: 1.2 mm and max: 3.1 mm). The buccal mucosa rapidly increases in thickness when moving apically. At 2.2 mm, thickness is 3 mm. No perceivable difference between the *Ti* and ZrO_2 abutment can be observed when thickness of the mucosa is 2 mm or more.

Conclusion: The difference in light reflection of soft tissue covering *Ti* or ZrO_2 abutments is no longer noticeable for the human eye when mucosa thickness exceeds 2 mm. Hemoglobin peaks in the reflection spectrum can be observed and make hyper-spectral imaging a practical and useful tool for measuring soft-tissue health.

Introduction

The ultimate challenge of restorative and implant dentistry is to replace all lost hard and soft structures, restore function and aesthetics, thus mimicking the unrestored, healthy tooth and its bony and soft tissue surroundings. With respect to the latter, the architecture, contour, surface texture and colour of the permucosal tissue are important determinants of the appearance of the restoration. Not much research is available with respect to the colour of the gingiva or peri-implant mucosa and its influencing factors, but it is presumed to depend primarily upon the intensity of melanogenesis, the degree of epithelial cornification, the depth of epithelialisation and the arrangement of gingival vascularisation (Dummett 1960; Kleinheinz et al, 2005). However, the colour of underlying root surfaces or restorative materials such as implant abutments, crown margins or even MTA are also considered to be of influence on gingival colour (Takeda et al, 1996; Jung et al, 2007; Bortoluzzi et al, 2007; Watkin & Kerstein 2008).

In the past, titanium abutments were the standard of care for implant restorations throughout the mouth. Unfortunately, blue-grayish shimmering of such abutments may hamper the aesthetic outcome in cases with thin overlying mucosal tissues and cause a noticeable colour difference with the gingival tissues of neighbouring teeth (Park et al, 2007). This is why initially alumina abutments were introduced but occasional fracture of abutments made from alumina was observed (Prestipino & Ingber 1993a; Prestipino & Ingber 1993b; Andersson et al, 2001; Andersson et al, 2003). The use of partially stabilized zirconia abutments has become more popular in recent years, especially in regions of high aesthetic demand (Watkin & Kerstein 2008). Such abutments combine high bending strength and toughness with good biocompatibility (Manicone et al, 2007). As with alumina abutments, the white colour of zirconia is considered aesthetically advantageous (Tan & Dunne, Jr. 2004; Glauser et al, 2004; Canullo 2007; Ishikawa-Nagai et al, 2007; Park et al, 2007; Bae et al, 2008).

It was demonstrated in vitro that the thickness of the overlying mucosa plays an import role on discoloration and the aesthetic appearance of soft tissues (Jung et al, 2007). In a randomized controlled clinical trial, spectrophotometric measurements at 1 mm below the gingival margin revealed no statistically significant differences in the discernable amount of discoloration between customized zirconia and titanium abutments with either all-ceramic or metal-ceramic crowns after 1 year (Sailer et al, 2009). The authors used a commercially available device originally intended for the determination of tooth shades. They stress the need for more controlled clinical trials to study the influence of the abutment material on the colour of the soft tissues. Indeed, criteria need to be defined to decide under what particular circumstances patients may benefit most from zirconia or titanium abutments. This is of interest, also considering the fact that the latter ones are usually more affordable and have a longer track record.

The present investigation focuses on the effect of zirconia and titanium implant abutments on light reflection of the supporting soft tissues in man, as it relates to the thickness of the

peri-implant mucosa. A novel method for the assessment of the colour of permucosal or gingival tissues is presented.

Materials and methods

A cross-over, within subject comparison study was designed.

Patient population and implant placement

Eleven consecutive Caucasian subjects scheduled to receive a total of 15 implants in the anterior region of the maxilla were included in the study after they had provided informed consent. Twelve out of 15 implant sites had been augmented prior to implant placement with autologous bone originating from the retromolar region. Soft tissue augmentations were not performed.

Under local anesthesia, a full thickness flap was raised with a crestal incision located approximately 2-3 mm toward the palatal aspect. Small relieving incisions were placed into the gingival sulcus of the adjacent teeth and extended to the mesio-buccal site of these teeth.

The palatal and buccal mucoperiosteal flap was elevated and the alveolar crest was inspected. When a bone augmentation procedure had been performed prior to implant placement, a small vertical incision was made in the mucosa overlying the bone graft at the position of the fixation screw. In this way the screw could be removed easily with little exploration and trauma, preventing disturbance of the vascularization.

A surgical template was used to assure proper placement of the implant. The implants (OsseoSpeed®, Astra Tech, Mölndal, Sweden) were 3.5 or 4 mm in diameter, placed at bone level, mostly in a position 1 mm apical to the cemento-enamel junction of the contralateral tooth. In all situations primary stability could be achieved. When the implants were placed in a submerged manner (7 implants), a cover screw was utilized. In all other situations (8 implants) a permucosal healing abutment of appropriate dimensions was placed (non-submerged healing). Wound closure was performed with Gore-Tex® sutures (W.L. Gore & Associates, Newark, DE) which were removed after 2 weeks.

Assessment of soft tissue thickness and height

At least 3 months after implant placement or, when applicable, at least 3 weeks after second stage implant surgery, the healing abutments were disconnected. A standard open tray impression registering the implant position and the surrounding soft tissues was made (Impregum, 3M Espe, Germany). Subsequently, an implant analogue was connected to the impression post and a plaster model was poured. The model was ground in a mesial-distal direction, in a plane parallel to the implant until the mid-buccal plane of the implant was reached (Figure 1). The specimen were photographed with a Canon EOS 20D photo camera with a 100 mm macro lens and a ring flash (8.2 mega pixels).

A line parallel to the implant was drawn. Perpendicular to this line, a series of lines was drawn with 0.2 mm intersections, starting at the most coronal point of the peri-implant mucosa. Along these lines the facial soft tissue thickness was measured in a commercially available computer program that allows image analysis (Adobe Photoshop CS3 extended). The images were calibrated, for which the known dimensions of (sections of) the implant analogue or a ruler that was photographed in the same plane were used.
In addition, the height of the permucosal tissues covering the implants was determined from the implant shoulder to the cervical margin (Figure 1).

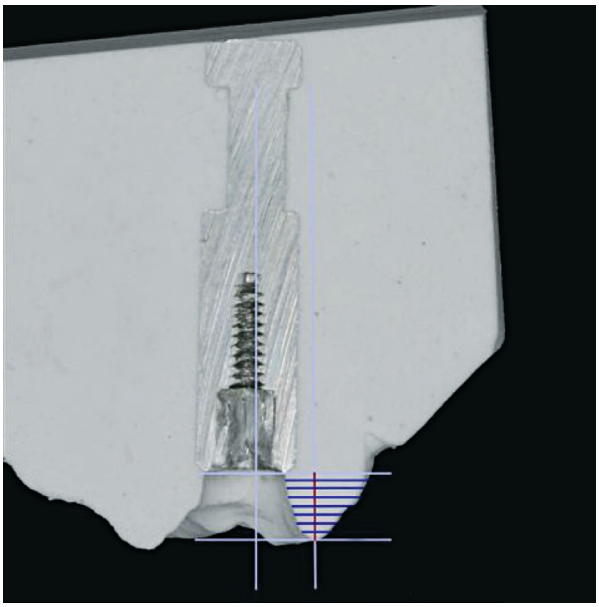

Figure 1: A plaster model is sectioned through the middle of the implant with reference lines for the measurement of soft tissue height and thickness. Soft tissue height is measured along the interrupted red line. Soft tissue thickness is measured at 0.2 mm intervals along the dark blue lines, commencing at the most caudal point.

Spectrophotometric measurements

Specially prepared dimensionally identical zirconia or titanium abutments were placed in random order. The dimensions of the permucosal section of these abutments were similar to those of the healing abutment. The abutments had been provided with markers to allow for calibration of linear measurements (Figure 2a-c). A time span of 15 minutes was allowed for settling of the permucosal tissues. A series of spectrophotometric measurements was made, the abutments were switched and the measurements were repeated.
High resolution images 1392x1024 were made with a hyper-spectral camera (Figure 3) (Noordmans et al, 2007b; Noordmans et al, 2009). Using an electronically tuneable optical bandfilter (vis-LCTF, Cri, Woburn, USA), images were captured from a wavelength of 440 to 720 nm with a stepsize of 4 nm. In this hyper-spectral image, the intensity $I_{n,t}(\chi, \gamma, \lambda)$ was determined for each pixel coordinate χ, γ and wavelength λ for implant *n* and abutment type *t*. Subsequently, the hyper-spectral images were corrected in two ways (Figure 4):

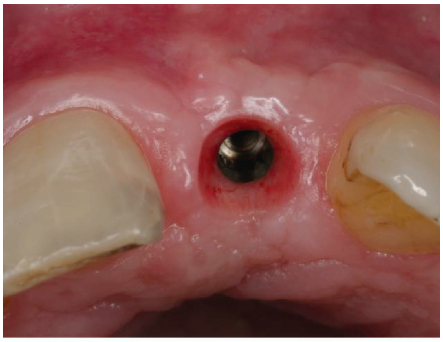

Figure 2a: Implant with healing abutment removed (patient nr. 6).

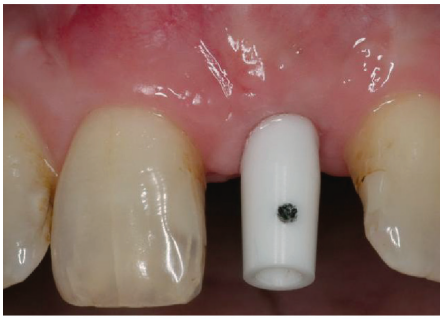

Figure 2b: Implant with the zirconia test abutment.

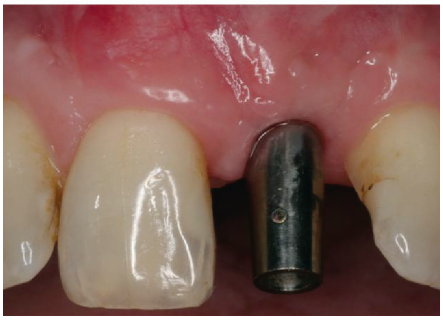

Figure 2c: Implant with the titanium test abutment.

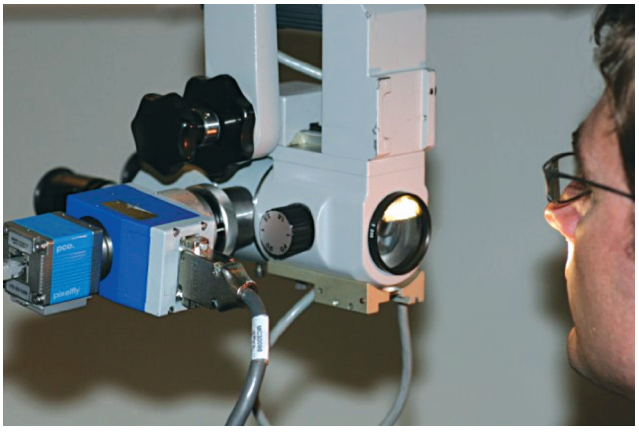

Figure 3: Experimental setup. The spectrophotometric device consisting of a high resolution monochrome camera (PCO) and a electronically tuneable colour filter (Cri) is connected to a microscope (Zeiss). A series of images is obtained, the abutment is switched and the procedure is repeated.

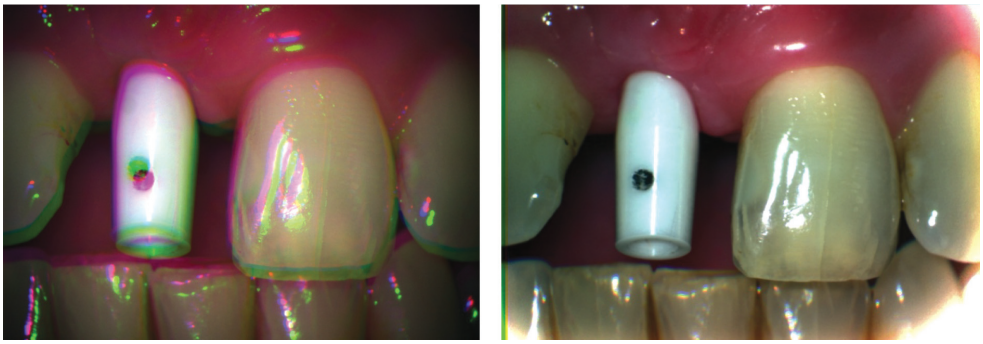

Figure 4: Hyper-spectral image normalisation to remove motion, and spatial and spectral inhomogeneities.

1. White balance and vignetting. By making a hyper-spectral image of both a white reference/object and in total darkness, the image can be corrected spatially and spectrally with the following formula:

$$R_{n,t}(x, y, \lambda) = \frac{I_{n,t}(x, y, \lambda) - D(x, y, \lambda)}{W(x, y, \lambda)} \qquad (1)$$

   Where *I* represents the captured image, *D* and *W* the dark and white reference and *R* the reflection image. To compensate for differences in distance between camera and subject, the reflection image was subsequently normalised by the reflection spectrum of zirconia, extracted 1 mm coronal of the mucosal margin.
2. Movement during acquisition and matching of the images. As the capture sequence takes about 10-30 seconds, small movements occur during acquisition. Fast GPU based image matching software was used to re-align the spectral slices based on rigid deformations and image correlation (Noordmans et al, 2007a). This software was also used to match the hyper-spectral images of the zirconia and titanium abutments.

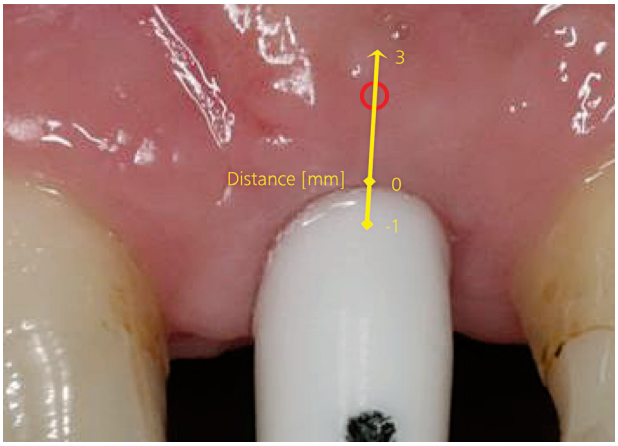

Figure 5: Definition of distance along central axis of abutment. Along this axis the reflection spectra are extracted. The circle denotes the averaged pixel spectra at each distance.

Spectra were extracted starting 1 mm coronal until 3 mm apical of the soft tissue margin in 0.05 mm steps (Figure 5). For each step, spectra were acquired in a small circular region to reduce noise and small highlights. The mean reflection spectrum along the zirconia and titanium abutment was then calculated by averaging over the spectra of all patients.
Subsequently, they were processed as follows. First, calibration of the distance axis along the central line was performed by setting the distance from the marker and the edge of the abutment to its real physical value. Second, the distance axis along the central line was converted to a mucosa thickness axis. Values for mucosa thickness *D* as a function of the distance to the cervical margin of the mucosa were obtained from the measurements on the plaster model. Then, the resulting spectra were converted to XYZ-colour space and finally to L*a*b* colour space using the following functions (Wyszecki & Stiles 2000):

$$\begin{bmatrix} X(D) \\ Y(D) \\ Z(D) \end{bmatrix}_{n,t} = \int_{\lambda} \begin{bmatrix} \bar{x}(\lambda) \\ \bar{y}(\lambda) \\ \bar{z}(\lambda) \end{bmatrix} \cdot R_{n,t}(D,\lambda) S(\lambda) d\lambda \Big/ \int_{\lambda} \bar{y}(\lambda) S(\lambda) d\lambda \qquad (2)$$

where $\bar{x}, \bar{y}, \bar{z}$ denote the colour matching functions and $S(\lambda)$ the spectral power density of a D50 light source.

$$\begin{pmatrix} L^*(D) \\ a^*(D) \\ b^*(D) \end{pmatrix}_{n,t} = \begin{pmatrix} 116 f_{Y(D)_{n,t}} - 16 \\ 500\left(f_{X(D)_{n,t}} - f_{Y(D)_{n,t}} \right) \\ 200\left(f_{Y(D)_{n,t}} - f_{Z(D)_{n,t}} \right) \end{pmatrix} \qquad (3)$$

$$\text{where } f_{P(D)_{n,t}} = \begin{cases} P(D)_{n,t}^{1/3} & \text{if } P(D)_{n,t} > 0.009 \\ \dfrac{903.3 \cdot P(D)_{n,t} + 16}{116} & \text{if } P(D)_{n,t} \le 0.009 \end{cases} \quad P \in \{X, Y, Z\} \qquad (4)$$

Results

Soft tissue thickness and height

The mean thickness of the buccal mucosa covering the abutment surface in the midline in relation to the distance from the cervical gingival margin is presented in Figure 6. The median value for the height of the mucosa to the edge of the implant is 2.3 mm (mean 2.4 s.d. 0.5 mm, min: 1.2 mm and max 3.1 mm).

Spectral analysis

As an example of the colour reconstructions, the processed images of patient 3 are presented following white balancing, vignetting, correction of movement and matching of the images of both abutment types (Figure 7). To enhance the difference in appearance of the mucosa, the images are divided by each other, resulting in a ratio image. A small, dark region in the

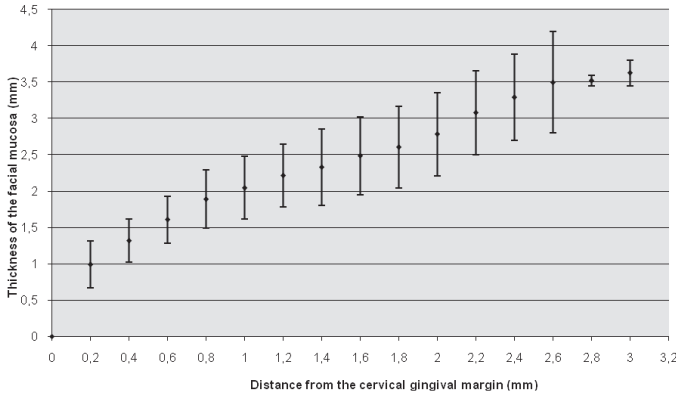

Figure 6: Mean facial soft tissue thickness (and standard deviations) in relation to the distance from the soft tissue margin at 0.2 mm intervals, n=15 implants in 11 patients.

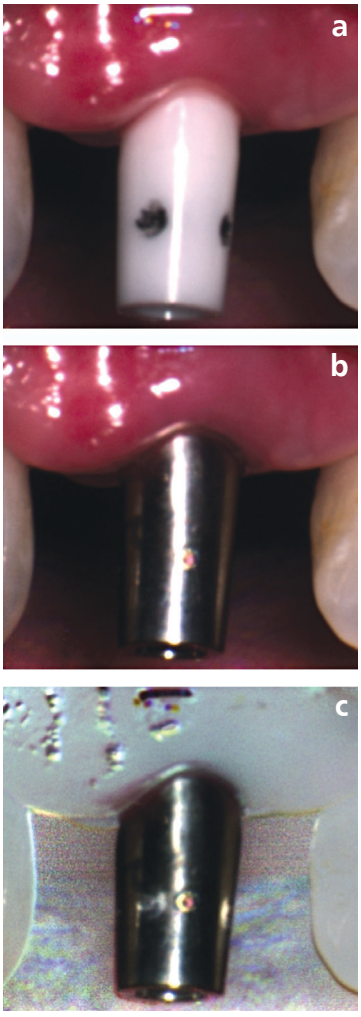

Figure 7: Difference in appearance of patient 3: a) Zirconia abutment, b) Titanium abutment, c) ratio image.

area of the emergence of the abutment can be perceived hinting that the influence of the abutment does not extend that far apically.

The mean spectrum at different distances from the mucosal margin for both abutment types is shown in Figure 8. Starting from *d*=-1 mm, the spectra still represents the spectrum of the abutment material itself, further apically the spectra become increasingly similar to that of mucosa tissue. Note that the absorption peaks of oxygenated hemoglobin are clearly visible.

To determine to which thickness of mucosa differences can be perceived by human observers, the distance axis is converted to a thickness axis using the measurements of Figure 6. The L*a*b* norms for both abutment types as a function of the mucosa thickness are shown in Figure 9. To determine at which thickness no difference can be perceived, the norm of the difference between the L*a*b* vectors is calculated and a threshold is set at a difference of 3.7 (Johnston & Kao 1989). This corresponds with a mucosa thickness of 1.95 mm.

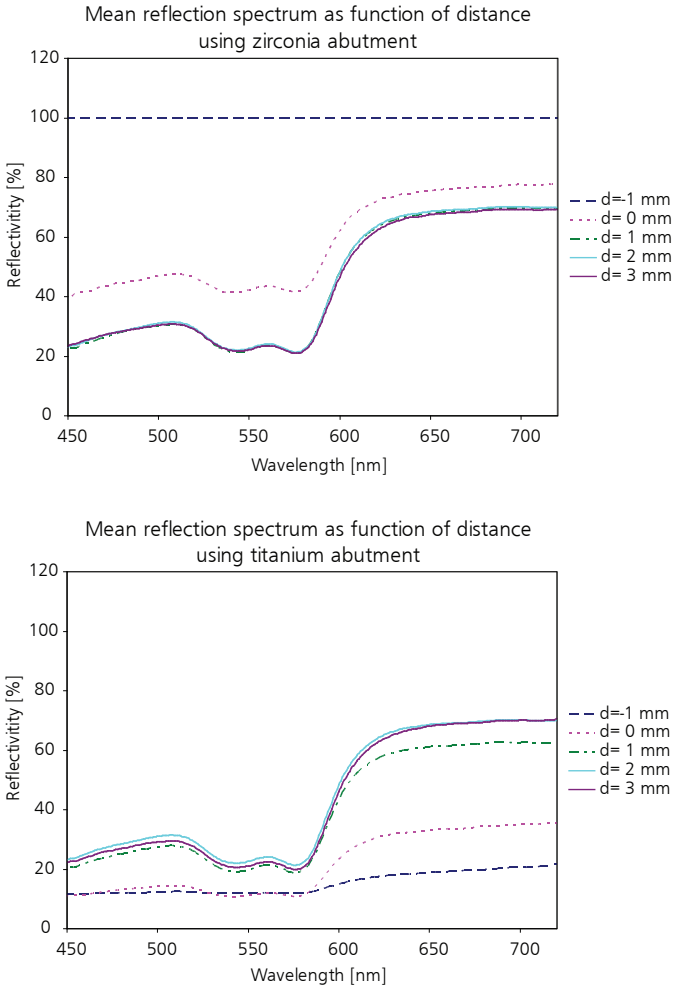

Figure 8: Mean reflection spectras of zirconia en titanium abutments, between 1 mm coronal of the cervical gingival margin and 3 mm apically. Note the particular shape of the reflection spectrum of oxygenated hemoglobin.

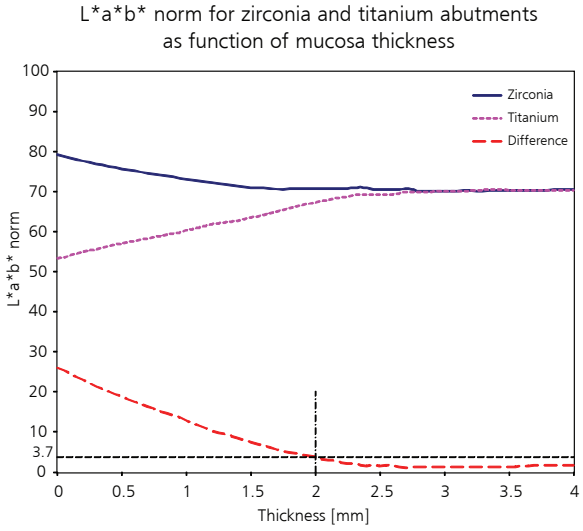

Figure 9: L*a*b* norms and difference as function of mucosa thickness to determine at which thickness no difference can be perceived.

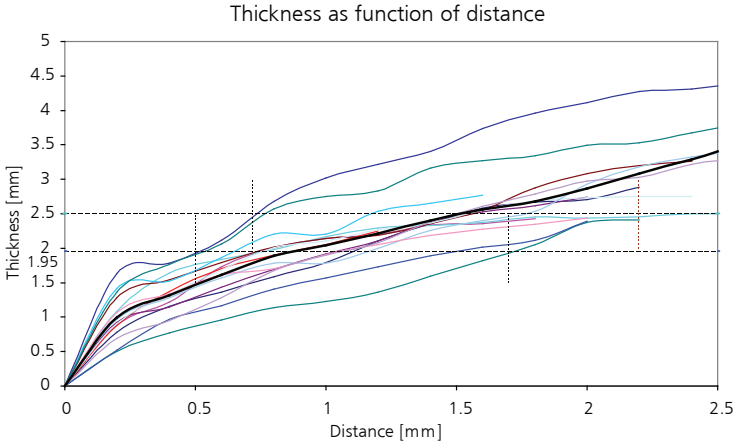

Figure 10: Figure 10: Variability in distance at which a difference between zirconia and titanium abutments can be perceived.

To see the effect of this thickness threshold on the distance at which differences may still be perceivable, the thresholds are transferred to the thickness/distance plots (Figure 10). One can notice that the range in distance is rather substantial: from 0.5 mm to 2 mm.

Discussion

The appearance of the permucosal tissue is an important determinant of the overall aesthetic outcome of an implant-borne restoration. It has proven difficult to mimick all aspects of the gingival appearance of the neighbouring teeth (Chang et al, 1999a; Belser et al, 2004; Furhauser et al, 2005; Belser et al, 2009). When the mucosa is thin and frail, it is prone for recession and underlying restorative materials will cause discoloration of the mucosa. Only little information is available regarding the dimensions, which is height and thickness, of the peri-implant mucosa in men.

Kan et al, measured the mid-facial height of the peri-implant mucosa in 2-stage anterior implants by means of bone probing with a periodontal probe after anaesthesia. They found an average height of 3.6 mm, with shallower values for subjects that were categorised as having a 'thin biotype' (Kan et al, 2003). In the present study in which also 2-stage implants were used, the median facial soft tissue height was 2.3 mm (minimum: 1.2 mm and maximum: 3.1 mm). It should be noted however that the height measurements on the plaster models reflect the height from the cervical mucosal margin to the edge of the implant, which is not necessarily also the location of the facial bone, although implants were originally placed 'at bone level'. Since the latter in most cases will be positioned more apically, this would explain the small difference with the clinical findings from Kan et al, It is interesting and clinically relevant to observe that when a 2-stage implant is installed at bone level, the maximum thickness of the overlying mucosa never exceeds 3.1 mm. This has implications for the ideal implant placement in relation to the neighboring teeth, especially with respect to the position of the implant shoulder. It underlines the clinical experience that implant placement too far below the cementum-enamel junction of the neighboring teeth will result in a non-harmonic soft-tissue architecture and a relatively long tooth, which will be difficult to correct.

From Figure 6 it can be observed that the mucosal thickness swiftly increases with the distance from the cervical gingival margin. At 1 mm apical from the cervical margin the mean thickness is approximately 2 mm. The maximum thickness seen in the 15 implant sites is 3.2 mm. Other studies also report on the thickness of the permucosal tissues around implants. Some used an ultrasonic device to measure the thickness of the mucosa at the bottom of the probeable pocket around an implant. Anesthetics were not used. They measured an average soft tissue thickness of 2 mm (Chang et al, 1999b). Although this seems an elegant non-invasive measurement technique, its reliability was never verified and the exact distance from the cervical margin was not disclosed. Others used endodontic files to measure the mid-facial soft tissue thickness at 1 mm apical to the margin. Jung et al report on 2.9 to 3.4 mm at all-ceramic and porcelain-fused to metal implant crowns respectively (Jung et al, 2008). In another report originating from the same research group the average soft tissue thickness around zirconia and titanium abutments was only 1.9 mm (Sailer et al, 2009). This corresponds well with the measurements from the present study, although an explanation for the difference with the study of Jung et al, is not given. Ishikawa-Nagai denote that they measured gingival thickness around implants in a study on the shine-through effects of

implants on the peri-implant mucosa, but don't report on the actual data (Ishikawa-Nagai et al, 2007).

Using a hyper-spectral camera may seem a bit of overkill to measure L*a*b* norm, especially since commercially RGB camera devices that obtain more or less comparable data are available. However, the current method can also be seen as a test case for measuring functional tissue properties like blood perfusion, tissue oxygenation and tracking of healing response or disease progression of infections or tumor growth (Sorg et al, 2005; Noordmans et al, 2007a; Noordmans et al, 2007b; Klaessens et al, 2009). For example, blood perfusion can be perceived by determining the amount of hemoglobin present in the reflection spectrum and oxygenation by looking at the difference between the oxy- en deoxygenated hemoglobin spectra. Not only does our method yield the spectral information along the central line of a tooth, but it also gives the full spectral information for the entire image so one could do tissue calculations for the entire image and assess the extent of tissue abnormalities.

Although the study shows a satisfactory result, a number of improvements may be included in the measurement setup: use of polarizer in the illumination path perpendicular to the polarizing axis of the tuneable filter to suppress specular highlights (Noordmans 2007b), perform a dark and white reference before each measurement, and include the ultra-violet range to study the fluorescence effects of abutments and crowns. Also, if one were able to measure the thickness of permucosal tissue of a sound tooth, one could compare the perceptible differences between permucosal tissue on sound teeth or that on implants. The used method to measure mucosa thickness on plaster models is not feasible for mucosa covering teeth.

Others have also focussed on the thickness of the mucosa as it relates to soft tissue aesthetics. Data from an in vitro experiment in sacrificed pig's maxillae using spectrophotometry suggest that when the thickness of the mucosa exceeds 3 mm, both titanium and zirconia do not cause a noticeable colour change of the mucosa (Jung et al, 2007). The authors used a Lab norms difference of 3.7, which is used in the literature as a threshold for perceivable colour differences (Johnston & Kao 1989). It would have to be determined how perceivabale differerences relate to clinical relevance or acceptability of a slight, but noticeable mismatch of mucosal appearance. From our in vivo data it can be expected that a noticeable difference between titanium and zirconia abutments occurs when the thickness of the facial mucosa is approximately 2 mm or less. There is considerable individual variance with respect to the depth at which this will be the case and therefore the choice of abutment material remains a decision that should be based on the clinical situation at hand. However, as a general rule of thumb, titanium implant abutments are best avoided in aesthetically critical areas when the desired location of the crown margin does not exceed 1 mm submucosally.

Conclusion

The labial mucosa covering an implant abutment rapidly increases in thickness when moving apically and is 1 mm thick at 0.2 mm, 2 mm thick at 1 mm below the cervical margin on average. The difference in light reflection between tissues covering zirconia and titanium implant abutments is no longer noticeable for the human eye when the mucosa thickness exceeds 2 mm. Hemoglobin peaks are clearly visible in the reflection spectrum which may render hyper-spectral imaging a practical and objective tool for monitoring soft-tissue health.

Acknowledgements

The authors wish to thank Mathieu Steijvers for the preparation of the plaster casts.

Chapter 6

General discussion and conclusions

This PhD thesis deals with issues related to the soft tissue response to zirconia (ZrO_2) and titanium (*Ti*) implant abutments and how these materials affect light reflection of the overlying mucosa. For each research project, the effort (time and money) spent in doing the study has to be justified. In this case, the limited amount of evidence from human studies comparing the relatively new ceramic material ZrO_2 with *Ti* in man was felt ample justification to undertake the project, for which permission by the medical ethics committee was granted. In particular human histology is hardly ever reported upon, but is presented in this thesis in chapters 3 and 4.

Each biomaterial that is used permucosally should facilitate peri-implant health (hence be biocompatiple) and have an adequate life cycle (hence be strong enough). In addition, implant abutments face the oral environment serving single or multi-unit tooth restorations and play a role in the esthetics, both the 'white' and 'pink'. The pink esthetics can be influenced by the color of the abutment if it is close to the gingival border (Jung et al, 2007; Park et al, 2007; Happe et al, 2013). Gray *Ti* may hamper aesthetics. White ZrO_2 may be advantageous.

For ZrO_2 to be able to compete with *Ti*, these aspects need to be researched. The primary goal of this thesis was to compare clinical, microbiological histological and optical aspects of ZrO_2 and *Ti* abutments. It was felt that the best study design for this would be a randomized clinical trial, with within-subject comparison (actually a split-mouth study). This study design has important advantages but also has some weaknesses. The major advantage is that it reduces error variance that is associated with individual differences. Much of the error variance in a between-subjects study design is due to the fact that individual factors influence the dependent variable, despite efforts taken to randomly assign subjects to groups. A within-subject study design increases power.

Weaknesses from a within-subject comparison study are potential carry-over effects (hence one treatment influencing the other). In our case, there seems to be no rational ground for concern for this to happen in both studies performed. Other concerns relate to compromised external validity. Recruitment of patients is hampered because of the need for symmetrical conditions requiring treatment, that are subsequently randomized. Restricting the recruitment to such patients in general limits the external validity of the results. In case of the present study this is of no great concern. In the Netherlands there are many edentulous subjects seeking implant overdenture treatment. Symmetrical allocation of implants is generally a prerequisite both for bar-retained and for bal-socket overdentures. Nevertheless it might bias the selection of patients towards those with a poorer oral hygiene rendering them edentulous, or perhaps smokers. The results from this PhD thesis are based on 2 studies, one focussing on the biological aspects, and one focussing on the optical aspects.

Zirconia versus Titanium implant abutments: biological aspects

When studying the biological aspects of ZrO_2 versus *Ti* as abutment material, the reactions of the tissues of the body on the abutment material and the behavior of the implant material

under the requested mechanical function in the body have to be evaluated and compared. With respect to the former, recent in vitro studies have shown that *Ti* implants provided with ZrO_2 abutments exhibit more internal wear than implants provided with *Ti* abutments (Klotz et al, 2011; Stimmelmayr et al, 2012; Cavusoglu et al, 2014). The biological ramifications from this finding, for instance the effect from transportation of *Ti* nano-sized particles to the soft tissues remain unclear and lie beyond the scope of this PhD thesis.

The tissue in direct contact with the abutment is mainly the gingival epithelium of the oral mucosa, but also the epithelium of the tongue, lips and cheeks. Since the gingival epithelium is the tissue in permanent direct contact with the abutment surface, the reaction of the epithelium on the two concerning abutment materials under investaigation is the main biological factor to be compared. This reaction is influenced by the surface properties of the abutment material and its mechanical properties enabling a narrow connection with the implant (without microbiological invasion) and enabling function without degradation of the material under long term occlusal loading. Also, possible adverse reactions on distance on the abutment material have to be taken into account. The function of the abutment has to be performed under occlusal forces generated by the person receiving the abutment, under his oral biochemical and oral microbiological conditions.

Favorable reactions of gingival oral epithelium on *Ti* as well as on ZrO_2 have been reported by several authors. Although some authors mention that the surface characteristics of ZrO_2 are more identical to those of the enamel of the natural human tooth, in the histological findings of this thesis, with results obtained in man, no significant differences with *Ti* are shown. Also for reactions on further distance of the abutment material and regarding clinical and microbiological conditions no differences were observed. This is confirmed by the most recent systematic review on the subject, which also included animal studies. From the 16 articles that were included in the review, after scrutinizing all initially selected articles, the majority found no difference in biological behavior between ZrO_2 and *Ti* abutments (de Medeiros et al, 2013).

The differences are therefore mainly to be found in the biomechanical properties of the two materials. *Ti* nowadays is the standard with its long studied, favorable biomechanical properties (strength, fatigue, resistance to degradation and wear, comparable properties of implant and abutment material), while ZrO_2 as a ceramic material by definition is sensitive to fatigue under long term loading and has different mechanical properties influencing the implant-abutment connection with risks of mechanical damage on implant or abutment, as stated above. So, from a standpoint of biological evaluation of the two materials, *Ti* may still be considered the standard, safe and best documented choice.

Zirconia versus Titanium implant abutments: optical aspects

When evaluating the optical aspects of the two materials, ZrO_2 is presumed to be advantageous. In cases where the margin of the implant abutment is supragingivally, be it directly after placement or after some time due to recession of covering tissues, a ZrO_2 abutment may be perceived as less disturbing by the patient than a *Ti* one. This is especially

true in the aesthetic zone and depending on the individual smile line. In addition, from the results of this thesis, it could also be concluded that in cases where the mucosa does cover the abutment, as it is usually intended, ZrO_2 is optically superior in cases with thin (that is < 2 mm) mucosa covering the abutment. This finding may influence abutment selection as discoloration of the mucosa is to be avoided whenever, possible in order to achieve optimal aesthetic results, mimicking (contralateral) natural teeth. Most other studies using spectrophotometry produced similar findings, although it should be realized that there is a difference between what can be measured instrumentally, what can actually be differentiated by the human eye and eventually how it is appreciated by the patient. With respect to the former, no clinical studies evaluating patient satisfaction with respect to esthetics in the anterior zone have demonstrated differences between ZrO_2 and *Ti* abutments as perceived by the patient (Bidra and Rungruanganunt, 2013).

In general, mechanical properties of ZrO_2 abutments are well suited to withstand occlusal loads especially in the anterior region where occlusal forces are the lowest. Only a few fractures, restricted to ZrO_2 implant abutments have been reported upon in this area (Bidra and Rungruanganunt, 2013) and application in this region seems justifiable in light of the optical advantages. More to the posterior, *Ti* abutments seem a more sensible choice.

The different mechanical properties between implant and abutment however will remain to be relevant, as possible leakage in the connection will make bacterial invasion possible, in time leading to possible gingival inflammation with negative consequences in both biological and optical respect. From a recent in vitro study it seems that under low torque values bacterial in- and efflux can be observed in systems with both abutment-types, with a smaller marginal gap seen in the *Ti* abutment specimens. However, increasing torque values decreased the gap of ZrO_2 implant abutments to the *Ti* implant considerably, suggesting a more intimate fit (Smith and Turkyilmaz, 2014).

Conclusions

With respect to the formulated questions of investigation it can be summarized that in general ZrO_2 and *Ti* abutment surfaces elicit similar clinical characteristics of peri-implant soft tissue health, microbiological and histological features during the first 3 months of function in man. Clinical and microbiological findings associate poorly with histological findings in relatively healthy permucosal conditions. Optically, differences in light reflection of the mucosa covering gray *Ti* and white ZrO_2 abutments can be distinguished by the human eye when the mucosa is thinner than 2 mm.

In the clinical field it is sometimes felt that implants restored with ZrO_2 abutments and all-ceramic crowns yield healthier soft tissues than when *Ti* implant abutments are used. The results from this study suggest that this assumption is probably not justified and that perceived differences are more likely to be optical than biological.

Regarding clinical relevance it can be stated that from a biological point of view, there appears to be not much difference in the soft tissue response to ZrO_2 and *Ti* abutments

and either can be used without preference. This conclusion is substantiated by a recent systematic review addressing this particular topic (de Medeiros et al, 2013). Care should be taken when interpreting conventional clinical parameters for monitoring the soft tissue condition in relatively healthy situations. Optically, ZrO_2 abutments may be favoured in aesthetically demanding cases where the overlying mucosa is thin. Interesting finding from the study on light reflection is the ability of the measurement technique to identify oxygenated hemoglobine peaks.

Suggestions for future research

- In light of the fact that clinical parameters of soft tissue health do not associate well with the histology as observed in this thesis, hyper-spectral imaging depicting hemoglobine peaks may prove to be a practical and objective tool for monitoring soft-tissue health. This technique should be further explored.

- With zirconia developments are still ongoing. In the past concerns were raised from the field of orthopaedics relating to loss of strength of the material over time as a result of a process called low temperature degradation or ageing when Yttria is used as a stabilizer. Whether this is of clinical relevance to dentistry as wel, has not yet been confirmed. Initial values for bending and flexural strength surpass the values of the tissues it is supposed to replace, but there is concern that it will weaken. Recently, different stabilizers among which Ceria and Alumina nanocomposites are presumed to be less effected by low temperature ageing degradation (Miyazaki et al, 2013). These might be materials for the future, requiring further testing.

- The connection of an abutment to the implant should be durable. Micro-movement and friction of zirconia abutments during clinical function might cause wear and tear of the inner part of titanium implants, but also of the abutment itself (Stimmelmayr et al, 2012; Cavusoglu et al, 2014). The wear products will be transferred to the vulnerable peri-implant environment, close to the bone. This subject, and other biomechanical aspects comparing zirconia and titanium abutments were not addressed in this PhD thesis but deserve critical appraisal.

Chapter 7

Summary in English

The establishment and maintenance of healthy soft tissues around implant abutments are important for long term service of the implant. The establishment of a stable and healthy permucosal seal that protects the underlying tissues from the intraoral environment depends heavily upon the adhesion, proliferation and colonization of fibroblastic cells and micro-organisms. Abutment surface properties, among which are biocompatibility (i.c. chemistry), surface topography (i.c. roughness) and surface-free energy are key influencing factors.

Titanium (*Ti*) has been the 'gold standard' material for implant abutments, but the use of high-strength ceramics is increasing. Zirconia (ZrO_2) is especially promising because of its high fracture toughness and favourable light dynamics. Little clinical data are available regarding the soft tissue reaction to ZrO_2, and how this compares to that of *Ti* in humans. The former is the general topic of interest of this thesis. More specific, 4 questions of investigation were formulated, comparing clinical, microbiological, histological and optical aspects between ZrO_2 and *Ti*-implant abutments:

1. Do ZrO_2 and *Ti* abutment surfaces exhibit similar clinical characteristics of peri-implant soft tissue health and microbiological features during the first 3 months of function?

2. Do soft tissues adjacent to ZrO_2 and *Ti* implant abutments exhibit similar histological aspects after 3 months of intraoral function?

3. Can clinical and microbiological parameters reflect soft tissue health around implant abutments when compared to histological observations, under relatively healthy conditions?

4. To what degree do ZrO_2 and *Ti* implant abutments effect light reflection of the supporting soft tissues in man?

For this PhD thesis, 2 clinical studies were designed and performed. The clinical, microbiological and histological issues are addressed in study 1 (chapters 2-4) and the optical aspects are addressed in study 2 (chapter 5).

Study 1: Clinical, microbiological and histological aspects and their associations.

Twenty edentulous subjects received 2 endosseous mandibular implants. The implants were fitted with either a ZrO_2 or a *Ti* abutment (non-submerged implant placement, within-subject comparison, left-right randomisation). Sulcular bacterial sampling and the assessment of probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were performed at 2 weeks and 3 months post-surgery. Statistical test were applied to test differences in the counts of 7 marker bacteria and the clinical parameters that were associated with the ZrO_2 and *Ti* abutments, at the 2 observation time points.

After 3 months of function soft tissue biopsies were obtained and prepared for histological evaluation. The specimen were subjected to blind evaluation per patient, both qualitatively and quantitatively. The number of blood vessels per surface unit was the primary outcome variable.
Finally it was investigated whether the clinical and microbiological findings as described in chapter 2 correlate with the histological findings in chapter 3. The former is considered to be the true state of soft tissue health, whereas the clinical and microbiological findings are more indirect indicators of peri-implant health.

Results from study 1

It was found that ZrO_2 and *Ti* abutments harbored similar counts of 7 marker bacteria at 2 weeks and 3 months. Healthy clinical conditions were seen around both ZrO_2 and *Ti* abutments at all times, without significant differences in most clinical parameters of peri-implant soft tissue health. Mean probing depths around *Ti* abutments were slightly deeper than around ZrO_2 abutments after 3 months (chapter 2).
Paired samples from 17 patients were deemed suitable histological evaluation after preparation. All specimen 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. No difference in vascular density between sites adjacent to ZrO_2 sites adjacent to *Ti* implant abutments were observed. They appear to elicit a similar soft tissue response in man (chapter 3).
Simple linear and linear regression models revealed that the clinical or microbiological parameters are not associated with the histological parameters. It was concluded that clinical and microbiological parameters associate not very accurate to histological parameters in conditions with relatively healthy peri-implant soft tissues in vivo.

Study 2: Optical aspects

Fifteen anterior implants (Astra Tech Implant System, OsseoSpeed™ implants, Dentsply Implants, Mölndal, Sweden) in 11 patients were fitted with a ZrO_2 and *Ti* abutment (crossover, within subject comparison). Hyper-spectral images were taken with a camera fitted on a surgical microscope. High resolution images with 70 nm interval between 440 and 720 nm were obtained within 30 seconds (1392x1024 pixels). Black- and white-point reference was used for spatial and spectral normalization as well as correction for motion during exposure. Reflection spectra were extracted from the image on a line mid-buccal of the implant, starting 1 mm above the soft-tissue continuing up to 3 mm's apically (chapter 5).

Results from study 2

Median soft-tissue height is 2.3 mm (min: 1.2 mm and max: 3.1 mm). The buccal mucosa rapidly increases in thickness when moving apically. At 2.2 mm, thickness is 3 mm. No

perceivable difference between the ZrO_2 and *Ti* abutment can be observed when thickness of the mucosa is 2 mm or more.
It is concluded that the difference in light reflection of soft tissue covering ZrO_2 and *Ti* implant abutments is no longer noticeable for the human eye when mucosa thickness exceeds 2 mm. Hemoglobin peaks in the reflection spectrum can be observed and make hyper-spectral imaging a practical and useful tool for measuring soft-tissue health.

Conclusions of this thesis in summary and clinical relevance

With respect to the formulated questions of investigation it can be summarized that in general ZrO_2 and *Ti* abutment surfaces exhibit similar clinical characteristics of peri-implant soft tissue health, microbiological and histological features during the first 3 months of function in man. Clinical and microbiological findings associate poorly with histological findings in relatively healthy permucosal conditions. Optically, differences in light reflection of the mucosa covering gray titanium and white zirconia abutments can be distinguished by the human eye when the mucosa is thinner than 2 mm.
Regarding clinical relevance it can be stated that from a biological point of view, there appears to be not much difference in the soft tissue response to ZrO_2 and *Ti* abutments and either can be used without preference. Care should be taken when interpreting conventional clinical parameters for monitoring the soft tissue condition in relatively situations. Optically, ZrO_2 abutments may be favoured in aesthetically demanding cases where the overlying mucosa is thin.

Chapter 8

Samenvatting in het Nederlands

Gezonde weke delen rond implantaatopbouwen zijn belangrijk voor de levensduur van het implantaat. Het bewerkstelligen van een stabiel en gezonde permucosale barrière die de onderliggende weefsels afschermt van het mondmilieu is afhankelijk van de adhesie, proliferatie en kolonisatie van fibroblasten en micro-organismen. Oppervlakte-eigenschappen van de opbouw zoals biocompatibiliteit (bijv. chemische samenstelling), oppervlaktetextuur (bijv. ruwheid) en vrije oppervlakte-energie zijn belangrijke factoren.
Titanium was de gouden standaard voor implantaatopbouwen, maar het gebruik van versterkte keramieken neemt toe. Zirconia in het bijzonder is veelbelovend vanwege de hoge breuktaaiheid en gunstig optische eigenschapppen. Er zijn weinig klinische data beschikbaar met betrekking tot de weke delen reactie op zirconia en hoe zich die verhoudt tot titanium in mensen. Dat vormde dan ook het algemene onderwerp van onderzoek in dit proefschrift. In het bijzonder werden 4 onderzoeksvragen geformuleerd ten aanzien van klinische, microbiologische, histologische en optische aspecten wanneer zirconia en titanium opbouwen worden vergeleken:

1. Worden rond zirconia en titanium implantaatopbouw oppervlakken vergelijkbare klinische en microbiologische karakteristieken gezien gedurende de eerste 3 maanden in functie?

2. Vertonen weke delen in contact met zirconia en titanium implantaat opbouwen een vergelijkbaar histologisch beeld na 3 maanden in functie in de mond?

3. Vormen klinische en microbiologische parameters een goede weergave van gezondheid van de weke delen als daarbij het histologisch beeld als standaard wordt genomen, in relatief gezonde omstandigheden?

4. In welke mate beïnvloeden zirconia en titanium implantaat opbouwen de lichtreflectie van bedekkende weke delen bij mensen.

Voor dit proefschrift werden 2 klinische studies ontworpen en uitgevoerd. De klinische, microbiologische en histologische vragen komen ter sprake in studie 1 (hoofdstuk 2-4) en de optische effecten in studie 2 (hoofdstuk 5).

Studie 1: De relatie tussen klinische, microbiologische en histologische aspecten.

Bij twintig edentate proefpersonen werden 2 enossale mandibulaire implantaten geplaatst. De implantaten werden voorzien van een zirconia of titanium opbouw (1 fase geplaatst, vergelijking binnen dezelfde patiënt, links-rechts gerandomiseerd). Plaquebemonstering uit de sulcus, pocketdiepte metingen (PPD), recessiemetingen (REC) en bloeding na sonderen (BOP) werden uitgevoerd op 2 weken en 3 maanden na plaatsing. Statistische testen werden toegepast om de verschillen in aantallen van 7 marker-bacteriën en de klinische parameters die werden geassocieerd met zirconia en titanium opbouwen te testen, op de

2 observatiemomenten. Na 3 maanden in functie werden weke delen biopten genomen en histologisch beoordeeld. De monsters werden blind geëvalueerd, zowel kwalitatief als kwantitatief. Het aantal bloedvaten per meetoppervlak was de primaire uitkomstmaat.
Tenslotte werd onderzocht hoe de klinische en microbiologische uitkomsten, zoals beschreven in hoofdstuk 2, zich verhielden tot de histologische uitkomsten, zoals beschreven in hoofdstuk 3. Voornoemde zou geacht kunnen worden de meest accurate weergave van weke delen gezondheid te zijn, daar waar klinische en microbiologische bevindingen een meer indirecte indicator vormen.

Resultaten van studie 1

Het bleek dat zirconia en titanium opbouwen vergelijkbare hoeveelheden van de 7 marker bacteriën herbergen na 2 weken en 3 maanden. Klinisch gezonde omstandigheden werden gevonden bij zowel zirconia als titanium opbouwen gedurende de hele studie, zonder significante verschillen in de meeste klinische parameters van peri-implantaire weke delen gezondheid. Gemiddelde pocketdiepte metingen rond de titanium opbouwen waren iets dieper dan bij zirconia opbouwen na 3 maanden (hoofdstuk 2).
Gepaarde testen bij 17 patiënten werden bruikbaar geacht voor histologische evaluatie na preparatie. Alle monsters vertoonden goed verhoornd meerlagig plaveiselcel epitheel, verlopend naar het aangehechte gingiva epitheel dat tegen het opbouwoppervlak lag. Een normale opbouw van het epitheel kon worden herkend. Er werd geen verschil in vaatdichtheid tussen de gebieden grenzend aan zirconia of titanium waargenomen. Er lijkt tussen beide materialen een vergelijkbare weke delen reactie bij mensen op te treden (hoofdstuk 3).
Eenvoudige lineaire en lineaire regressiemodellen onthullen dat de klinische en microbiologische parameters niet geassocieerd zijn met de histologische parameters. Geconcludeerd werd dat klinische en microbiologische parameters niet accuraat aansloten bij de histologische parameters in omstandigheden met relatief gezond peri-implantaire weke delen in vivo.

Studie 2: Optische aspecten

Vijftien fronttand implantaten (Astra Tech Implant System, OsseoSpeed™ implants, Dentsply Implants, Mölndal, Sweden) bij elf patiënten warden voorzien van een zirconia en titanium opbouw (kruislings, binnen een en dezelfde patiënt vergeleken). Opnamen werden gemaakt met een hyperspectrale camera gemonteerd op een chirurgische microscoop. Hoog-resolutie opnamen met een interval van 70 nm tussen 440 nm en 720 nm werden verkregen in 30 seconden (1392x1024 pixels). Zowel zwart-, en witbalans referentiepunten werden gebruikt voor ruimtelijk en spectrale normalisatie als correctie voor beweging tijdens de opname. Reflectiespectra werden verkregen van een lijn mid-buccaal door het implantaat, beginnend 1 mm boven de gingiva tot 3 mm apicaal op de afbeelding (hoofdstuk 5).

Resultaten van studie 2

De gemiddelde weke delen hoogte is 2,3 mm (min: 1,2 mm en max: 3,1 mm). De dikte van de labiale mucosa neemt snel toe in apicale richting. Op 2,2 mm van de gingivarand is de dikte 3 mm. Geen waarneembare verschillen tussen zirconia en titanium opbouwen kunnen worden gevonden als de bedekkende mucosa meer dan 2 mm dik is.
Geconcludeerd kon worden dat verschillen in lichtreflectie van de weke delen die zirconia of titanium opbouwen bedekken niet meer kunnen worden waargenomen als de mucosa meer dan 2 mm dik is. In het reflectiespectrum kunnen hemoglobinepieken worden waargenomen. Die maken hyperspectraal opnamen mogelijk een praktisch en bruikbaar middel om weke delen gezondheid te meten.

Conclusies uit dit proefschrift samengevat en klinische relevantie

Met betrekking tot de onderzoeksvragen kan worden samengevat dat in het algemeen weke delen op zirconia en titanium opbouw oppervlakken vergelijkbare klinische karakteristieken vertonen qua gezondheid, microbiologische samenstelling en aantallen bacteriën en histologische uitkomsten, gedurende de eerste 3 maanden in functie bij mensen. Klinische en microbiologische uitkomsten associëren matig met histologische bevindingen in relatief gezonde permucosale omstandigheden. Optisch gezien kunnen verschillen in lichtreflectie van de mucosa, die grijs titanium of wit zirconia opbouwen bedekken worden waargenomen als de mucosa dunner is dan 2 mm.
Met betrekking tot klinische relevantie kan worden gesteld dat er uit biologisch oogpunt niet veel verschil in weke delen reactie tussen zirconia en titanium opbouwen is, en dat beiden wat dat betreft zonder voorkeur kunnen worden gebruikt. Voorzichtigheid moet worden betracht als conventionele klinische parameters worden gebruikt bij het beoordelen van de conditie van de weke delen in relatief gezonde omstandigheden. Ze geven mogelijk niet een juiste indruk. Optisch zijn zirconia opbouwen in het voordeel bij esthetisch veeleisende gevallen, als de bedekkende mucosa dun is.

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Curriculum vitae

Ralph van Brakel was born on March 17 1965 in Delft, the Netherlands. In 1984 he completed pre-university secondary education (VWO) at the Huygens Lyceum in Voorburg. That year he started dental school at the "Academisch Centrum Tandheelkunde Amsterdam" or better known as ACTA, which he completed in January of 1990.

After working in several dental practices he started a solo-practice in Hoofddorp in 1991 and was a co-worker in a dental office in Voorschoten. In September 1999 he stopped in both offices and started a joint dental practice with two colleagues in Wassenaar on the day his daughter Juliet was born. In 2005 he also accepted a part time position at the department of Oral & Maxillofacial Surgery and Special Dental Care at the University of Utrecht Medical Centre. During this time he completed the requirements for maxillofacial prosthodontist (as recognized by the Nederlandse Vereniging voor Gnathologie en Prothetische Tandheelkunde "NVGPT") in 2009.

In collaboration with prof. dr. Marco Cune and dr. Jan Willem Verhoeven the research was initiated in 2007 that is the base for this thesis.

Ralph van Brakel is married to Carien Daniels and they have a daughter, Juliet (1999) and a son, Thom (2001).

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