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Soft tissue response to different abutment materials: A controlled and randomized human study using an experimental model

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Conflict of Interest

The study was founded by Schilli-Implantology-Circle (Basel, CH), C+M (Biel, CH) and the authors' own institutions. The authors declare that they have no conflict of interest.

Author contribution

N.E.: Investigation, methodology, project administration, conceptualization, funding acquisition, supervision

M.M.: Formal analysis, visualization, data curation, writing – original draft, writing-review & editing

S.B.: Resources, supervision

W.G.: Data curation

M.S.: Data curation, writing-review & editing

D.K.: Resources, methodology, conceptualization, writing-review & editing, formal analysis, visualization, supervision

Abstract

Objectives

Aim of this study was to compare the soft-tissue response to implant abutments made of titanium, zirconia, zirconia veneered with feldspar ceramics and PEEK by various clinical, histological, microbiological and molecular biological markers in an experimental model.

Materials and Methods

40 experimental one-piece healing abutments of four different materials were mounted on bone-level implants in 20 volunteering patients (split-mouth design). After a three months period of open healing,

clinical parameters at the abutments were assessed and adjacent mucosa was sampled for inflammatory cytokine mRNA-concentrations and histological analysis by a novel method. In addition, PISF-samples were obtained for the analysis of periodonto-pathogenic bacteria counts and active MMP-8 levels. Marginal bone level change was measured by intra oral radiographs.

Results

Abutments of the different materials did not exhibit significant differences regarding clinical parameters, pathogenic bacteria counts or pro-inflammatory cytokine concentrations. Likewise, no significant differences were detected regarding soft tissue morphology or bone level change. Compared to titanium abutments, significantly less mononuclear inflammatory cells were detected in the mucosa at abutments made of zirconia veneered with feldspar ceramics.

Conclusions

All examined abutment materials exhibited a similar soft tissue response compared to titanium and histological data did not reveal early signs of elevated inflammation caused by PEEK- and feldspar-veneered zirconia abutments. Due to the short observation period and the small sample size, a final conclusion on the long term suitability of those abutment materials cannot be drawn. However, based on the presented data, we consider further studies on that subject as appropriate.

Keywords: *peri-implant soft tissue, soft tissue response, soft tissue integration, histological analysis, abutment materials, titanium, zirconia, feldspar ceramics, PEEK*

MeSH: Dental Abutments, Gingiva, Dental Implants, Dental Materials, Randomized Controlled Trial

Introduction

The abutment constitutes the transmucosal part of dental implants and links the osseointegrated anchorage to the dental superstructure. After implant insertion, the keratinized peri-implant mucosa forms a seal-like barrier around the abutment that protects the underlying bone from insults of the intraoral environment. Characterized by a tight attachment of epithelia and connective tissues, soft tissue

integration of abutments is of fundamental importance to long-term implant stability and success. Effective soft tissue integration and an appropriate soft tissue response, in turn, are highly dependent on the abutment material used.

Nowadays, predominantly abutments made of titanium are used in clinical restorations because of their high biocompatibility and the excellent long-term success rate (Zembic et al., 2014). However, even with advanced implant designs, there are features -especially regarding the quality of the mucosal seal- that differ significantly between gingival and peri-implant tissues. Moreover, commonly used titanium abutments exhibit a grayish color that remains visible after submucosal installation and might thus impact the esthetic implant outcome, especially when placed in the anterior region with a thin mucosal biotype (Cosgarea et al., 2015; Lops et al., 2017). To meet the esthetic challenges in the visible domain, the use of zirconia abutments has been established as a viable alternative for some time. Because of its white color, zirconia abutments create a more natural appearance (Naveau et al., 2019) that is esthetically advantageous in mucosa with a thickness of less than 3 mm. Zirconia, just like titanium, is highly biocompatible and has demonstrated a high success rate in long-term clinical studies (as reviewed in Cao et al., 2019).

On the other hand, the idea of using materials like poly-ether-ether-ketone (PEEK) for permanent dental restorations is an emerging concept in prosthodontics. Even though PEEK has already been successfully employed in the field of non-dental implantology (Kurtz & Devine, 2007), the exposure to the non-sterile intraoral environment might have an effect on microbial interactions and soft tissue response that have not yet been investigated in detail. PEEK abutments exhibit good esthetic- and functional properties; the color of PEEK is close to that of human teeth, it is biocompatible and has a similar elastic modulus when compared to that of human bone (Garcia-Gonzalez et al., 2015). However, PEEK abutments are predominantly used for provisional restorations (Papathanasiou et al., 2020) as there are few studies on the long-term success and as the majority of available studies on the dental soft tissue response are carried out *in vitro*.

A further approach to employ experimental implant abutments is by the usage of well-established materials serving as abutment basic structure with subsequent modification of the surface properties by staining or veneering. (Happe et al., 2013). But likewise, the direct or indirect effects of those modifications on the peri-implant soft tissues are rarely (e.g.: Thoma et al., 2018) investigated in human trials.

For gaining functional insights in the process of soft-tissue response to different materials, the conduction of comprehensive human histological studies is considered inevitable (Vignoletti & Abrahamsson, 2012; Tomasi et al., 2014.). However, the extraction of suitable, standardized samples for histological analysis is technically challenging and might interfere with the overall implant success. As a result, the majority of controlled studies on soft-tissue response to different abutment materials has been performed in a pre-clinical context (e.g.: Welander et al., 2008; Rea et al., 2017; Caballé-Serrano et al., 2019) or only include clinical parameters, which on their own are limitedly suited as surrogate markers for implant success (van Brakel et al., 2014; Coli & Sennerby, 2019) –particularly at early time points.

Even though several controlled trials report histological data on the soft-tissue interactions of the most commonly used materials like Titanium vs. Zirconia (Arvidson et al., 1996; Degidi et al., 2006; van Brakel et al., 2012; Schwarz et al., 2013; Sampatanukul et al., 2018), the amount of studies comparing novel abutment materials is not satisfactory. To date, for example, there is only one pilot study in humans, in which a study protocol for the controlled and randomized histological comparison of multiple abutment materials is introduced - including non-standard materials like PEEK (Borie et al., 2020).

Thus, in this controlled experimental human study we compared the soft-tissue interactions and response to experimental one-piece abutments made of i) titanium, ii) zironium dioxide, iii) zirconium dioxide veneered with feldspar ceramics and iv) PEEK, after three months of open healing. We applied an experimental model to obtain non-separated histological specimens of the abutment-mucosa interface, as previously described in Kraus et al., 2021. To complement the histological data, clinical parameters, marginal bone level change, microbiological load and cytokine mRNA tissue levels at the different abutment materials were evaluated three months after abutment installation.

Materials and Methods

Study design

A controlled prospective human study was conducted to evaluate the soft-tissue interactions for different abutment materials. Experimental healing abutments of 4 different materials (i)Titanium [grade 5 titanium, SIC invent AG, Basel, Switzerland], (ii)Zirconia [CAD/CAM crafted, Heraeus Kulzer GmbH, Hanau, Germany], (iii)Zirconia veneered with Feldspar ceramic [Veneering: Labor Ampellio, Bern, Switzerland] and (iv)PEEK [PEEK CLASSIX®, Invibio Ltd., Thornton Cleveleys, United Kingdom] were specifically

designed for this study. The hollow-cylinder shaped abutment prototypes exhibited lateral openings in the cylinder wall (2.3 mm in width) that allowed for the adjacent mucosa to grow inside and attach to the inner cavity of the abutments. The windows were positioned at the level of the abutment baseplate, covering the transmucosal portion of the abutments beyond the mucosal margin (For visual illustration of the experimental abutments, see: Kraus et al., 2021). Experimental abutments were mounted on epicrestally installed SICace® titanium implants (SIC-Invent, Basel, Switzerland) in the interforaminal region of the mandibles in 20 edentulous patients (t0), two different abutments each (n=40). After three months of open transmucosal healing (t1), marginal bone level changes around the implants were recorded by intraoral X-ray films, clinical parameters were assessed and peri-implant sulcular fluid (PISF) samples were obtained for microbiological examination. Mucosal tissues that had grown into the abutment's cavity were sampled together with the abutment for histological analysis. Avoiding full paramarginal excision, the section was limited to the area of the transmucosal openings (Scalpel No. 15), followed by the unscrewing of the non-separated specimens with a slotted screwdriver.

Soft tissue response after 3 months was evaluated in the non-separated mucosal tissues regarding the following primary endpoint variables:

(i) Histological parameters: Inflammatory mononuclear- and neutrophil cell counts in ingrown mucosa; Quality of collagen fibers; Quality of rete pegs. See Figure 1 for exemplary grading series.

(ii) Clinical parameters: visible inflammation and suppuration, bleeding on probing (BoP) and probing depth (PD)

Additionally, the secondary outcome variables: (iii) marginal bone level change, (v) inflammatory cytokine profile, (vi) periodonto-pathogenic bacterial load and (vii) Plaque-index (PI) were assessed.

This experimental study was part of a regular definitive implant therapy that involved a single surgeon (N.E.) at the School of Dental Medicine (University of Bern, Bern, Switzerland). The study was conducted in accordance to the guidelines of Helsinki (2013 Brazil) as well as along the European directives, the ICH Harmonized Tripartite Guideline E6: Note for Guidance on Good Clinical Practice, CPMP/ICH/135/95 Step 5 (http://www.ema.europa.eu/ema/) and the CONSORT guidelines. The study protocol for the clinical part had been reviewed and approved by the Cantonal Ethics committee of Bern, Switzerland, 157/2008.

For clarity, we will refer to "*zirconia veneered with feldspar ceramic"* as "*feldspar"* in this study.

Study population

20 volunteering edentulous patients (64.6±10.1 years old; 8 males, 12 females; Table 1) were recruited at the School of Dental Medicine at University of Bern and provided informed consent. Eligibility criteria included a good general health status and sufficient bone height in the anterior mandibles, without preceding bone regeneration procedures. Exclusion criteria were pregnancy or nursing, a present infectious disease, osteoporosis or use of drugs that influence bone metabolism, mucosal changes, heavy smoking (>10 cigarettes /day) or an extraction in the anterior mandibles that was less than six months ago.

Table 1: Baseline demographics: Patient Age [years] at the time of abutment installation, sex and abutment assignment.

Subject	Age	Sex	Material 1	Material 2
1	66	f	PEEK	Ti
2	68	f	PEEK	Fsp
3	81	m	Fsp	Zir
4	69	f	Fsp	Ti
5	84	m	Ti	PEEK
6	49	m	Fsp	PEEK
7	59	m	Zir	Ti
8	69	m	Ti	Zir
9	54	f	Fsp	Ti
10	68	f	Zir	PEEK
11	66	f	Fsp	Zir
12	50	f	Zir	PEEK
13	77	m	PEEK	Zir
14	73	f	Zir	Ti
15	57	f	Ti	Fsp
16	50	f	PEEK	Fsp
17	55	f	Zir	Ti
18	72	m	Fsp	PEEK
19	58	f	Ti	PEEK
20	66	m	Fsp	Zir

Clinical procedure

A full thickness flap was raised in the interforaminal region of the mandibles and overall, two titanium implants were inserted in region 33 and 43 according to the standard protocol. Experimental abutments were immediately mounted to the epicrestally placed implant-shoulders (t0), according to the randomization plan. Drilling protocol and all surgical prodecures were carried out under local anesthesia

(Ubestesin forte, Epinephrine 1:100.000, 3M-Espe, Seefeld, Germany). After abutment installation, the flaps were adjusted, fitted to the abutment inner cavity to ensure optimal contact, and sutured. Post-operative care included a mouth rinse with 0.2% chlorhexidine gluconate (Meridol perio, GABA, Therwil, CH) twice a day for one minute in the following week and gentle cleaning with soft specialty brushes (TePe Compact Tuft, TePe Munhygienprodukter AB, Malmö, Sweden). One week post-op, sutures were removed and abutments were left to heal for three months in an open healing position. After three months (t1), peri-implant mucosa was inspected for clinical parameters, standardized intraoral control radiographs were taken and PISF was probed with sterile paper points. The abutments were subsequently harvested along with the ingrown mucosal tissues by excision in the area of the abutment openings (Scalpel No. 15), followed by unscrewing of the specimens. All sampling procedures were conducted by the same investigator (N.E.). The applied harvesting method avoids paramarginal circular excision around the abutment and can thus be considered less invasive than existing harvesting protocols. During the healing period, a provisional full denture was mounted. After the sample acquisition, patients were subjected to definitive restoration.

Randomization and allocation concealment

Allocation of the different experimental abutment combinations was concealed to the surgeon until abutment installation. For within-subject comparison (split-mouth) a permuted block randomization list was generated, containing a set of six possible material combinations (Ti:Zir, Ti:Fsp, Ti:PEEK, Zir:Fsp, Zir:PEEK, Fsp:PEEK). Within one block (block size of 6), numbers 1 to 6 were randomly assigned to the material pairs by a computer generated list and concealed in opaque envelopes. At abutment installation, before the respective envelope (drawn in ascending order) was opened, two coin flips ensured left-right randomization and the random chronological order of installation.

Clinical parameters

At (t1), peri-implant mucosa was inspected for visible inflammation and suppuration. Bleeding-on-probing and Plaque-index (O'Leary et al., 1972) were assessed at the mesial, buccal, distal and oral aspects of the implants. Peri-implant probing depth was determined by the mean distance from the mucosal margin to the pocket base at 4 aspects per implant, using a periodontal probe.

Radiographic examination

Intraoral radiographs were recorded at baseline after implant insertion (t0), and immediately prior to the sampling procedure after three months (t1). The radiographs were taken in a paralleling technique, by the use of customized film holders. Regions of interest on the intraoral films were digitalized with a 20-fold

magnification and analyzed (DBS-Win 4.5; Dürr Dental AG, Bietigheim-Bissingen, Germany). Measurements were calibrated by implant length and marginal bone levels were determined by the averaged distance of the first bone-to-implant contact to the implant shoulder at the mesial and distal aspect of the implants. The pictures were independently examined three times by two trained dentists, each. If the differences in measurements among the examiners were 0.1 mm or less, the mean of the independent measurements was used. If the differences were greater than 0.1 mm, the specific film was re-analyzed together and consensus was sought.

Microbiological evaluation and aMMP-8 concentrations

Peri-implant sulcular fluid (PISF) was sampled after three months (t1) for microbiological analysis and sulcular active MMP-8 concentrations, on consecutive days. The peri-implant mucosa was cleaned and dried with cotton pellets to prevent contamination with saliva. The sampling procedure was conducted with sterile paper points (ISO #90, Roeko, Langenau, Germany), that were inserted in the peri-implant sulcus for 30s. The paper points for characterization of sulcular active neutrophil collagenase levels (aMMP-8) were transferred to sterile tubes and sent to a blinded laboratory for quantification by ELISA (Dentognostic GmbH; Jena, Germany), as previously described (Munjal et al., 2007). The paper points for microbiological analysis by DNA-DNA Checkerboard hybridization were processed in the Department of Periodontology (University of Bern, Bern, Switzerland) as described elsewhere (Socransky et al., 2004). Microbiological evaluation comprised the detection of DNA from 6 periodonto-pathogenic bacteria that was quantified (Image Quant, Amersham Pharmacia, Piscataway, NJ) by comparison to DNA standard lanes, extracted from 10^5 and 10^6 bacterial cells. The detection level by this method is provided as $0.75*10^4$ bacteria per sample for each of the target species.

mRNA expression levels

At abutment disconnection (t1), small tissue fragments of the attached mucosa were harvested at the distal aspect of the implant for measurement of tissue mRNA-expression levels. Tissue specimens were directly snap frozen in liquid N_2 and stored at -80 °C for further processing. Total RNA was isolated from mucosal tissue using the RNeasy® Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) and was quantified by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). First strand cDNA synthesis was performed with the iScript™ cDNA synthesis kit according to the manufacturer's protocol (Bio-Rad Laboratories, Munich, Germany) using 1 µg of total RNA input. The real time qPCR (CFX Connect™ Real-Time PCR System; Bio-Rad Laboratories, Munich, Germany) was performed by adding cDNA equivalent to 50 ng RNA to a master mix containing gene-specific primers (Metabion, Martinsried,

Germany) and iQ™ SYBR® Green Super-mix (Bio-Rad Laboratories, Munich, Germany). PCR conditions were as follows: An initial activation step 95 °C / 5 min was succeeded by [95°C / 15 s denaturation, primer-specific annealing temperature (AT) °C / 30 s, and 72 °C / 30 s for elongation]^{x50}. Relative differential gene expression was calculated using the ΔΔCt-method (Pfaffl, 2001), normalized to the titanium material group and with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as internal housekeeping gene. For sequences, ATs and efficiencies of IL-1β, IL-6, IL-8 and IL-10 specific primers see Kraus et al., 2012.

Histology

Sections of the Specimens containing the non-separated mucosal tissues together with the abutment were stained by toluidine blue (see Figure 2 for overview pictures) and were subsequently analyzed in an optical microscope (ZEISS Axioskop 2®; Carl Zeiss AG, Carl-Zeiss-Straße 22, 73447 Oberkochen). See (Kraus et al., 2021) for detailed protocol. In brief, ground sections (Donath and Breuner, 1982; Rohrer and Schubert, 1992) were produced by fixing the non-separated samples in 4 % formalin, followed by dehydration in an ascending ethanol series (70 %-100 %) and two step resin infiltration (TECHNOVIT 7200 VLC®; Kulzer GmbH, Hanau, Germany) for a total of 14 days in a dark, low pressure environment. The hardened blocks were cut by a diamond band saw (200 µm; EXAKT® 300CP) and attributed to grinding (EXAKT® 400CS) and polishing.

Pictures (ZEISS AxioCam MRC®) of the sections (40 µm) at primary magnifications ranging from 1.6x to 50x were digitalized as JPEG files (axioVision Rel.4.7). Quantitative analysis of the inflammatory cells was performed by counting mononuclear inflammatory cells and neutrophils in the connective tissues at 50x magnification; epithelial areas were excluded from quantification. A total of three characteristic areas (200 µm x 280 µm) were investigated per sample. Semi-quantitative parameters *Quality of collagen fibers* and *Quality of rete pegs* were assessed at 1.6x to 25x magnification and ranked on a three grade scale. The histological evaluation was performed by a blinded anatomist (W.G.).

Analysis

Primary objective was the comparison of the four different abutment materials regarding the following outcome parameters, at (t1):

-Mononuclear- and neutrophil cell counts in the ingrown connective tissues [cells per 0.056 mm^2, examined in three characteristic areas, two times each].

-Quality of collagen fibers (1=few/loose; 2=average/wavy; 3=strong/strand-like) at 10x and 25x magnification in the connective tissue.

-Quality of rete pegs, regarding the degree of epithelial interlocking (1=weak; 2=average; 3=high) at 1.6x and 10x magnification.

-Clinical parameters: bleeding-on-probing [binary decision], visible inflammation [binary decision] and probing depth [mm].

Secondary outcome variables included the comparison of a wide range of microbiological-, molecular biological and radiographic data between the material groups at (t1):

- Cytokine mRNA expression levels of IL-1β, IL-6, IL-8 and IL-10 in the peri-implant mucosa by qPCR normalized to GAPDH.

- Abundance of the six putative periodonto-pathogenic bacteria A. *actinomycetemcomitans*, F. *nucleatum*, P. *gingivalis*, P. *intermedia*, T. *denticola* and T. *forsythia*, referenced to bacterial standards (10^5; 10^6); Cumulative values.

- Concentration of active neutrophil collagenase (aMMP-8) in peri-implant sulcular fluid [$ng*ml^{-1}$].

-Marginal bone level changes (t1-t0) around the implant [mm].

- Plaque-index [0-4, presence of visible plaque at four examined sites]

Estimation of sample size

Sample size estimation was based on the detection of group differences in mononuclear inflammatory cell counts between Titanium, Zirconia, Feldspar and PEEK abutments (log values; ANOVA Design). Probability of Type I error was set to 0.05. Standard deviations were estimated based on data collected in a previous feasibility study (Kraus et al. 2021) and were set to 0.24. For estimation of relevant differences in inflammatory cell numbers between different materials, the available comparative human data was searched. However, the severity of inflammation was predominantly specified qualitatively or semi-quantitatively via grading scales. The inflammatory cell number in inflamed periodontal tissues may be increased by magnitudes when compared to healthy gingiva (see Dutzan et al., 2016; Fine et al., 2016).

Due to its sensitive nature, an easy to identify effect size was chosen, that allows for possible inferences on the long term performance of the materials (0.31 log difference, roughly reflecting a 2 fold change in inflammatory cell numbers). To achieve 80% Power, a sample size of 10 abutments per material group was calculated (G*Power 3.1.9.7; Faul et al., 2007).

Statistical Analysis

For statistical analysis, log values of the parameters: *Mononuclear* and *neutrophil cell counts*, *aMMP8 levels* and *Abundance of perio-pathogenic bacteria* were used. Statistical analysis of primary and secondary outcomes was performed in SPSS (Version 27, IBM, Armonk, USA). Generalized mixed models were fitted to account for the split-mouth design, with *patients* as subject variable (random effect) and *abutment material* as fixed effect. If variance attributed to the random effect was redundant, subject variable was omitted in the model. For analysis of the parameters: *Mononuclear inflammatory cell counts* (log), *Neutrophiles* (log), *aMMP8* (log), *Abundance of perio-pathogenic bacteria* (log), *bone level change* and *PD*, a linear mixed model was chosen. For ordinal outcomes: *Quality of Rete pegs*, *Quality of Collagen fibers* and *Plaque Index*, ordinal regression mixed model with cumulative logit link was used. *BoP* was modelled with a binary logistic regression mixed model. Differences between the material groups were compared separately for all dependent variables and p-values were not adjusted for testing multiple outcomes. For pairwise comparisons of the fixed effects, confidence intervals were adjusted with sequential Bonferroni. Estimations are based on the Kenward-Roger approximation for small sample sizes. Graphs were created by GraphPad Prism 5.02 (GraphPad Software, San Diego, USA). Results are noted as Values [lower 95% CI , upper 95% CI] or ±SD.

Results

Clinical observations

A total of n=20 Patients (40 abutments) fulfilled the eligibility criteria and were included in this study. Due to the particularly thin-walled design of the experimental healing abutments (see Figure 2), 8 experimental abutments in 5 patients were lost during the healing phase, either due to fracture (Zi: 2; Fsp: 2) and deformation (Peek: 3), or because of visible mucosal detachment from the abutment (PEEK: 1). All data relating to those samples were excluded from analysis. Two abutments (Zir: 1; Fsp: 1) fractured during the sampling procedure at abutment disconnection, in a way that soft tissues were not suitable for further analysis. All patients complied with oral hygiene measures and exhibited healthy peri-implant conditions with no visible inflammation or suppuration at the time of abutment disconnection. No

significant differences between the material groups were detected regarding PI (P = 0.421) (Table 3), BoP (P = 0.378) and PD (P = 0.142). Estimated means or Medians of clinical and histological primary outcomes are summarized in Table 2. Firm attachment of the peri-implant mucosa to the abutment was present in all included samples when clinically examined. However, abutment-mucosal attachment was lost in a majority of the samples after histological processing.

A slight marginal bone level recession was observed for all abutment materials after the three months healing period *(t1-t0)*. Differences in bone level change between the material groups were small (Mean difference < 0.11 mm ± 0.39 mm) and did not exhibit a statistical significance (P = 0.796; Table. 3).

*Table 2: Effects of different abutment materials on the primary outcome parameters after 3 months of open healing. Estimated means of Mononuclear inflammatory cells and Neutrophils [log counts*0.056 cm^{-2}]; BoP [% of positive findings]; Probing depth [mm]. Median and Range of Collagen quality and Quality of Rete pegs [1-3]. Lower and upper 95% Confidence Intervals are indicated in brackets.*

Abutment	log Mononucl.			log Neutrophils			BoP			PD			Collagen			Rete pegs		
	Mean	*n*	*CI [95%]*	*Mean*	*n*	*CI [95%]*	*Mean*	*n*	*CI [95%]*	*Mean*	*n*	*CI [95%]*	*Median*	*n*	*Range*	*Median*	*n*	*Range*
Titanium	1.95	10	[1.77 , 2.14]	0.5	10	[0.34 , 0.67]	16.1	10	[3.1 , 53.7]	2.54	10	[2.25 , 2.82]	2.5	10	2	2	10	2
Zirconia	1.86	7	[1.64 , 2.08]	0.61	7	[0.41 , 0.81]	53	8	[17.2 , 86]	2.27	8	[1.97 , 2.57]	3	7	2	2	7	2
Feldspar	1.53*	7	[1.31 , 1.75]	0.35	7	[0.15 , 0.55]	52.1	8	[16.3 , 85.8]	2.16	8	[1.86 , 2.47]	3	7	1	2	7	2
PEEK	1.88	6	[1.64 , 2.12]	0.39	6	[0.18 , 0.61]	53.4	6	[14 , 89]	2.44	6	[2.10 , 2.78]	2	6	2	2.5	6	2

**p<0.05; referred to Titanium abutments*

Histological observations

General peri-implant soft-tissue health, as evaluated by the quality of rete pegs, was similar between the different abutment materials (P = 0.659). Assessment of collagen fibers in the proximity of the abutment-mucosal interface did not reveal significant statistical differences between the material groups (P = 0.304).

Analysis of the inflammatory infiltrate evaluated at 50x magnification did not demonstrate significant differences in the neutrophil cell counts between the material groups (P = 0.252). Neutrophil cell counts in feldspar- and PEEK abutments were actually distributed slightly lower as compared to titanium and zirconia abutments (Figure 3a; Table 2). For mononuclear inflammatory cells, that made up the vast majority of the inflammatory infiltrate, a significant difference between the material groups was detected ($F(3, 26) = 3.337$; P = 0.035; Figure 3b), as the cell count was reduced almost three fold at feldspar abutments when compared to titanium abutments (fold reduction: 2.65 [1.06 , 6.59]; P = 0.032; seq. Bonferroni adjusted pairwise comparisons).

Cumulative load of selected pathogenic bacteria in PISF

Of the six examined periodonto-pathogenic bacteria, *F. nucleatum* was most frequently detected in the PISF around 25 implant sites (in more than half of all samples), followed by *A. actinomycetemcomitans* (in more than one third of all samples). The presence of *P. gingivalis*, by contrast, was not detected in any patient. Mixed effects model did not reveal a significant influence of material choice on the cumulative periodonto-pathogenic baterial load in the peri-implant sulcular fluid (P = 0.203; Table 3).

Active MMP-8 concentrations and Interleukin mRNA-levels

Active MMP-8, an early marker for periodontitis and peri-implantitis, was successfully analyzed in the PISF around 23 implants. According to an estimated cut off value of 8 $ng*ml^{-1}$ indicative for a healthy gingiva (see Meissen et al., 2014 for threshold considerations), none of the investigated samples exhibited signs of tissue breakdown. PEEK abutments in average exhibited higher sulcular aMMP-8 levels than the other material groups, whereas Feldspar abutments scored the lowest values (Table 3). Differences between the groups failed to reach statistical significance (P = 0.11). Likewise, the analysis of the of mucosal IL-1β-, IL6-, IL8- and IL-10 mRNA expression levels did not reveal significant differences in the expression profile when compared to titanium after three months of open healing (All P-values > 0.5; data not shown).

*Table 3:Effects of different abutment materials regarding secondary outcome parameters after 3 months of open healing. Estimated means (Mean),Medians and number of analyzed sites (n) of the following targets are indicated: Bone level change (ΔBL_{t0-t1}) [mm]; active MMP-8 [log ng*ml^{-1}]; Pathogenic load [log Abundance (counts*10^4) of DNA from 6 periodonto-pathogens]; 95% Confidence Intervals are indicated in brackets. Median and Range are specified for ordinal Plaque-index [number of positive findings per abutment; 0-4].*

Abutment	ΔBL_{t0-t1}			log aMMP-8			log Pat. Load			PI		
	Mean	*n*	*CI [95%]*	*Mean*	*n*	*CI [95%]*	*Mean*	*n*	*CI [95%]*	*Median*	*n*	*Range*
Titanium	0.44	10	[0.2 ; 0.67]	0.39	7	[0.14 ; 0.63]	0.00	9	[-0.40 ; 0.39]	1.5	10	2
Zirconia	0.42	8	[0.15 ; 0.68]	0.11	5	[-0.18 ; 0.39]	0.62	6	[0.14 ; 1.11]	1.5	8	4
Feldspar	0.57	8	[0.31 ; 0.83]	0.04	5	[-0.25 ; 0.33]	0.46	6	[-0.02 ; 0.94]	2.5	8	4
PEEK	0.49	6	[0.18 ; 0.79]	0.5	6	[0.23 ; 0.76]	0.2	4	[-0.39 ; 0.79]	1	6	2

**p<0.05*

Discussion

Aim of the present study was to deploy a previously introduced model of less invasive mucosal sampling for the comparison of abutment materials. Beside histological evaluation, which can be considered as highly sensitive indicator for soft tissue response, the analysis of four different materials was complemented with a multitude of surrogate indicators for implant success.

The amount of human histological studies that directly assess and compare the soft tissue interactions with experimental abutment materials is sparse, whereas scientific research in that field is urgently needed to improve the understanding of soft tissue integration of implant abutments for long term implant success. Just recently the group of Bacevic et al. published a first pilot study that compared multiple experimental abutment materials regarding soft tissue integration and additional outcomes at the histological level (Borie et al., 2020). In their pilot study, a tissue punch was used for paramarginal mucosal excision around experimental abutments. The group directly sampled and imaged the mucosal-abutment interface in a separating technique to obtain conclusive results, whereas our model primarily focuses on the reduction of tissue damage during the sampling of mucosa that is ingrown to the abutments' inner cavity. Our model addresses the demand for human histological studies that otherwise would not be feasible due to ethical restrictions, and can thus be considered a trade-off between patient safety and scientific significance.

Titanium, Zirconia, Feldspar (veneer) and PEEK abutments were compared regarding soft- and hard tissue responses - with an emphasis on the performance of experimental PEEK and feldspar abutments. The results suggest an acceptable soft tissue reaction towards the tested materials, as there were no prominent negative effects detected, when compared to titanium after 3 months of open healing. PEEK abutments exhibited a higher failure rate in our study that is, however, due to the thin-walled experimental abutment design.

Soft tissue response and inflammation

The soft tissue response of all examined abutment materials was acceptable after three months of open healing, as the experimental materials neither induced visible signs of inflammation, increased peri-implant probing depths nor increased marginal bone level recession. Available data on the BoP at titanium- and zirconia abutments that indicate lower BoP-values for zirconia (reviewed in Sanz-Martín et al., 2018 and Sanz-Sánchez et al., 2018) could not be corroborated in our study, possibly due to the short healing period combined with the small sample size.

In general, inflammation of the peri-implant mucosa is relatively common around dental implants (Koldsland et al., 2010). Peri-implant inflammatory infiltrates often display a chronic state of inflammation (Degidi et al., 2012; Thoma et al., 2018) that is characterized by mononuclear inflammatory cells (CD20+; CD3+; CD68+), whereas mucositis is associated with elevated mononuclear cell counts in the sub-sulcular connective tissues (Sanz et al., 1991). Other studies however, reported on higher proportions of neutrophils in the inflammatory infiltrate in healthy (Broggini et al., 2003; in dogs) and inflamed (Carcuac & Berglundh, 2014) peri-implant sites. In the results presented here, neutrophil cell counts, which reflect acute inflammation, represented only a small proportion of inflammatory cells in all material groups (5.3%±3.1) and a significant difference regarding acute inflammation between the groups was not detected. Concerning neutrophil- and mononuclear-driven inflammation at zirconia abutments, our results are in accordance with previous studies that found similar or slightly less inflammatory infiltrates around zirconia tissue level implants in the connective tissues (reviewed in Roehling et al., 2019) and the barrier epithelium (Wenz et al., 2008) when compared to titanium. However, mononuclear inflammatory cells -which accounted for the vast majority of detected immune cells- exhibited significantly lower counts in the mucosa at veneered feldspar abutments in our study, whereas a previous human study comparing zirconia abutments and porcelain veneered zirconia abutments at the histological level found no difference in the inflammatory reaction after 6 months (Thoma et al., 2018). Comparative data on the inflammation around human PEEK abutments is hardly found in the literature. A study from 2019 in pigs

discovered significantly more MNGCs in the proximity of healing caps made of PEEK when compared to titanium after 4 and 8 weeks (Caballé-Serrano et al., 2019); a finding that was not confirmed in our study after 3 months of healing.

Formation of a soft tissue seal

In previous studies that compared titanium and zirconia abutments or tissue level implants, soft tissue integration and anatomical dimensions of the peri-implant mucosa has been considered similar in a preclinical- (reviewed in Roehling et al., 2019) and human context (van Brakel et al., 2012; Thoma et al., 2018). For feldspar ceramics and PEEK on the other hand, the amount of data on soft tissue integration is unsatisfactory to date. Compared to titanium, PEEK abutments exhibited similar mucosal dimensions in dogs (Rea et al., 2017), as confirmed by histomorphometry, and similar dimensions in human when assessed by clinical parameters alone (Koutouzis et al., 2011). Feldspar porcelain fused to gold exhibited less mucosal attachment compared to titanium (Abrahamsson et al., 1998) in one preclinical study. Our data suggests no significant differences between titanium-, zirconia-, feldspar- and PEEK abutments regarding the quality of collagen fibers in the abutments' proximity, even though slightly weaker collagen fibers were observed in the proximity of PEEK abutments (Table 2). The informative value of this isolated parameter, however, is certainly not sufficient for a conclusive statement on soft tissue integration.

Marginal bone level changes

Marginal bone level change after implant installation is a potent and commonly utilized indicator for implant success (Misch et al., 2008). The majority of marginal bone loss occurs shortly after implant insertion and may amount to 2 mm within the first year. Marginal bone loss around zirconium abutments and titanium abutments seems to be equivalent or slightly in advantage for zirconium abutments when compared after one- to five years in function (Linkevicius & Vaitelis, 2015; Sanz-Sánchez et al., 2018; Hu et al., 2019), whereas conflicting data exist on the bone loss around PEEK abutments: In a human study on PEEK abutments, Koutouzis et al. reported no increase in bone loss after a 3 months healing period when compared to titanium abutments (Koutouzis et al., 2011), but another study reported significantly increased bone recession around PEEK in a pre-clinical study after 4 months of healing (Rea et al., 2017). In the results presented here, only minimal differences in bone level change were observed across the material groups. While the parameter is presumably too robust for revealing subtle differences between the materials in such a short time frame, the results do not indicate any severe hard tissue reactions towards PEEK or feldspar abutments.

Pro-inflammatory cytokines and active MMP-8

The expression of inflammatory cytokines IL-1β-, IL6-, IL8- and IL-10 in the peri-implant mucosa did not reveal any significant differences between the material groups after three months of open healing. The concentration of active MMP-8 in the PISF, an established early marker for the detection of mucositis and periodontitis (Meissen et al., 2014) was consistently scored lower than 8ng*ml^{-1} in all groups, with only a slight increase around PEEK abutments.

Plaque index and abundance of selected periodonto-pathogenic bacteria

Plaque accumulation and pathogenic bacterial infection is considered as main cause for the onset of mucositis and peri-implantitis. We monitored the abundance of periodonto-pathogenic bacterial DNA in the peri-implant sulcus around the abutments to link those findings to other early markers for implant success. Pre-clinical and experimental human studies on the bacterial load of titanium and zirconia dental materials suggest that zirconia may impede bacterial colonization in experimental settings (e.g. Nascimento et al., 2014). However, in other *in vitro* studies (de Avila et al., 2017) and in a clinical context this effect is often not observed around implant abutments (Salihoğlu et al., 2011). For PEEK abutments and veneered feldspar abutments, there is a lack of available data in this regard. So far, *in vitro* studies suggest a low bacterial colonization of PEEK in the oral realm (Hahnel et al., 2015; D'Ercole et al., 2020). In our study, no significant differences were detected between the four dental materials regarding the plaque index or the cumulative abundance of the 6 putative periodonto-pathogenic bacteria A. *actinomycetemcomitans*, F. *nucleatum*, P. *gingivalis*, P. *intermedia*, T. *denticola* and T. *forsythia* in PISF. In future experiments, however, more attention should be drawn to the surface characteristics of the materials, as they might have a larger effect on initial bacterial colonization than materials themselves (Huang &Huang, 2019). Raster electron microscope pictures of the here employed abutment surfaces are attached in the supplementary files (Figure 4).

General limitations

In future studies, the presented model can be readily modified for reduced sample thickness and subsequent immunostaining, which cannot be achieved in non-separated, resin embedded samples. However, there are inherent factors that limit the informative value of the model: The presented model does not reliably reflect histomorphometric properties of the peri-implant mucosa. The inspected tissue portion is not directly linked to the underlying bone and tissue orientation is often convoluted due to the confinement of the enclosing abutment cylinder. The model's direct informative value regarding soft

tissue integration is thus limited to the notion of collagen quality in the abutment-mucosa interface and the presence of mucosal adherence. Moreover, the thin-walled experimental design relies on lateral openings that further compromise the overall structural integrity of the abutments. Excessive or non-uniform pressure by the superimposed provisional dentures may induce considerable loading stresses: Six of the forty initially placed abutments in this study failed to survive the three months healing period due to fracture or deformation and consequently had to be excluded from analysis. The frequent loss of valuable patient data, however, results in a demand for higher subject numbers in return, which conflicts with the advantages of a less invasive sampling method and is opposed to the initial objective of our model. To limit patient risk, we therefore closely monitored the abutments for early signs of structural failure. As the structural abutment properties are expected to have an increased effect with time, we do not recommend the implementation of the model for longer time periods. Another limiting factor that is inherent to the abutment design is the cleanabilty of the implant site. Compared to standard healing abutments, cleaning and plaque removal is more cumbersome in our model and might lead to an increased between-subject variability, based on individual oral hygiene. We therefore extensively trained patients in cleaning with soft dental brushes and quantified oral hygiene via Plaque index after the healing period.

While the presented results do not allow for reliable statements on the long-term performance of the examined materials, they reflect the soft tissue response to the dental materials after the initial healing process is completed and may thus facilitate decisions on their suitability for further use in long term clinical studies. The statistical evaluation of the presented data is only of secondary importance here, as for many parameters other than the inflammatory cell count, a substantially higher patient number would be required for meaningful statistical power. We think however, that our study can make a contribution to the yet limited knowledge of human material-soft-tissue interactions.

Conclusion

In this experimental human study, we examined the soft tissue response to four different abutment materials after 3 months with a multitude of clinical, histological, as well as micro- and molecular biological indicators. Despite the small sample size and the limitations of the applied model, the results suggest an acceptable soft tissue reaction for both zirconia abutments veneered with feldspar ceramics and PEEK abutments. Especially the results of veneered feldspar abutments are promising and indicate that feldspar fused to zirconia might exhibit favorable traits when compared to standard dental materials.

In our opinion, the promising results can provide a basis for new studies on the long term performance of the two materials.

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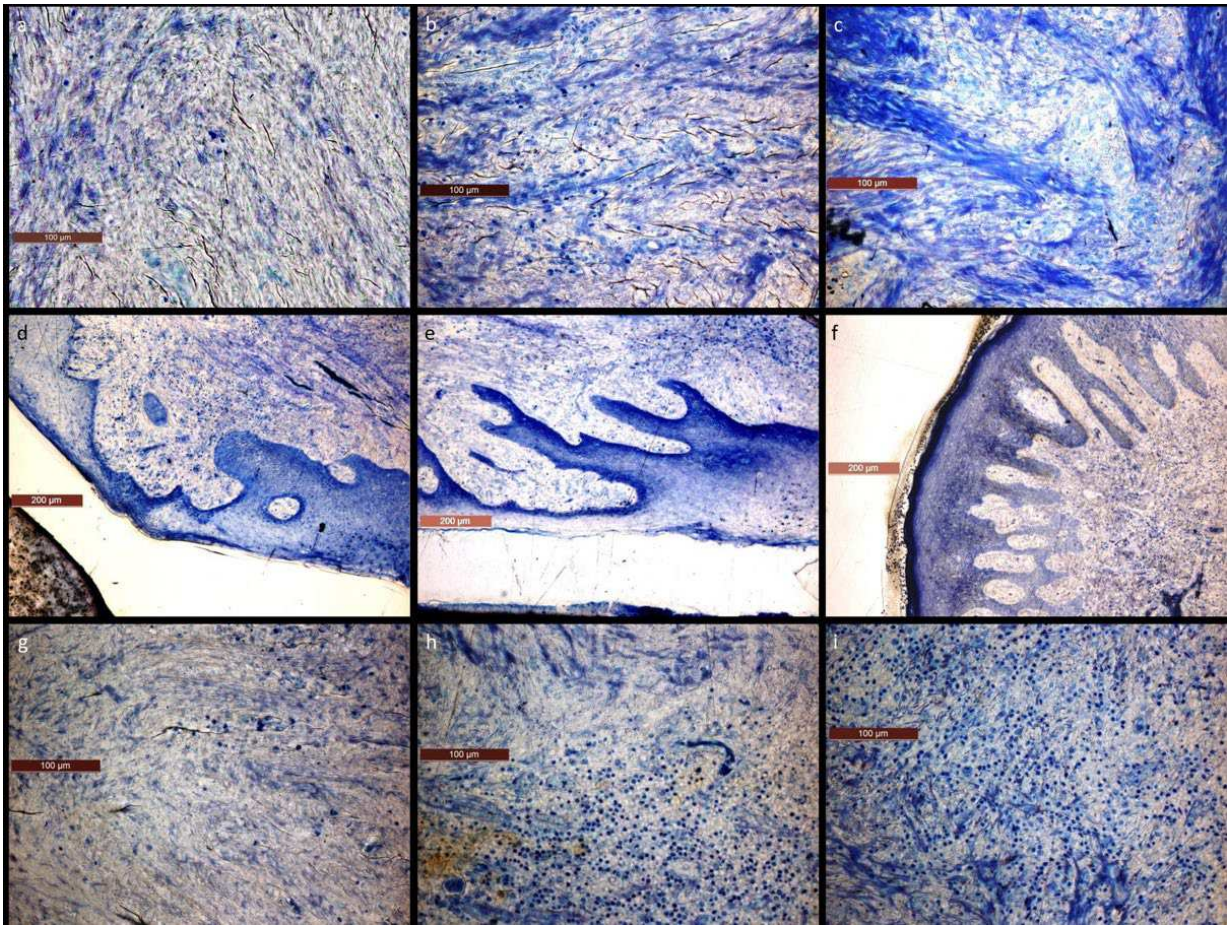

Figure 1: Exemplary toluidine blue picture series for indicators of per-implant soft tissue response in the presented model. (a-c): Quality of collagen fibers (a = few/loose; b =average/wavy; c =strong/strand-like). (d-f): Quality of rete pegs (d = weak; e = average; f = high). (g-i): Inflammatory infiltrates of increasing severity as observed at 25x primary magnification in the connective tissues.

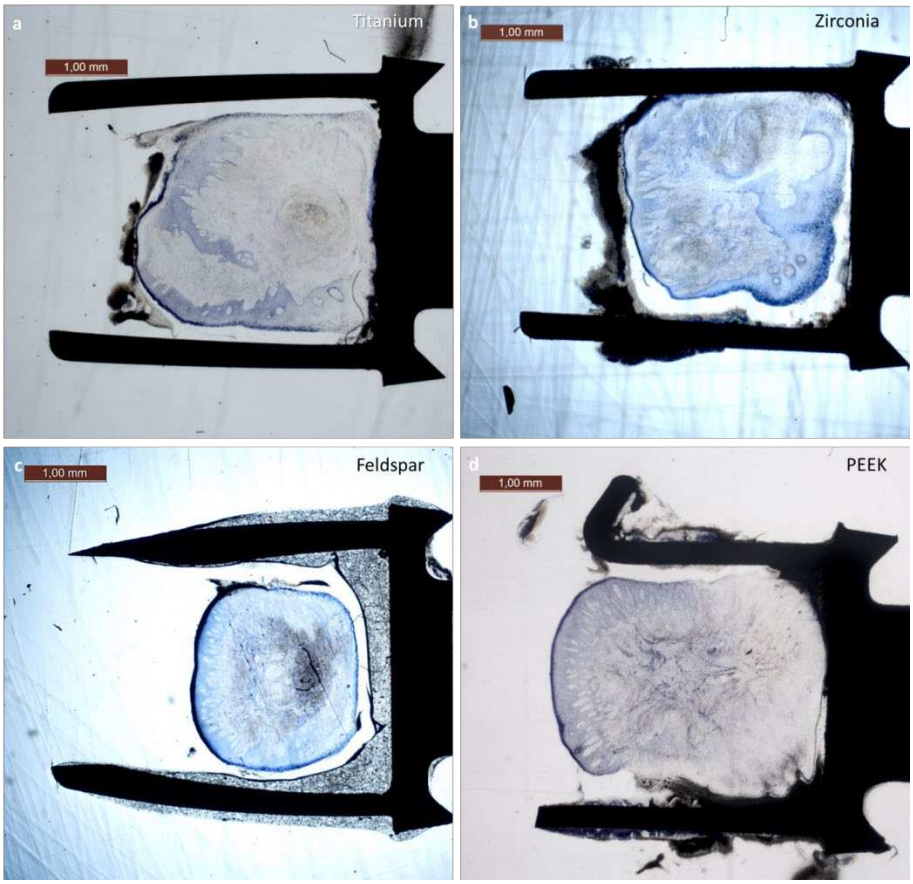

Figure 2: Representative histological pictures of the different abutments, together with the ingrown soft tissue portion after 3 months of open healing. Mucosal adhesion to the abutment wall was frequently lost after processing due to shearing forces (b) or tissue shrinkage (c). The veneer is clearly visible for feldspar abutments (c); deformation of the abutment wall can be observed at the PEEK-abutment (d).

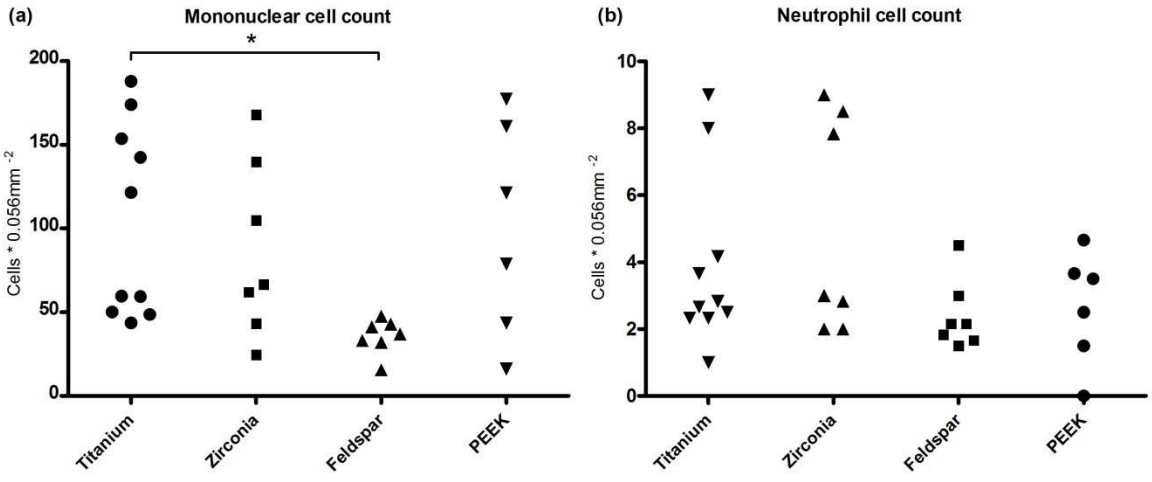

Figure 3: Comparison of connective tissues at experimental titanium-, zirconia-, zirconia veneered with feldspar ceramics- (Feldspar) and PEEK abutments regarding mononuclear cell infiltration (a) or neutrophil granulocyte infiltration (b).