Early Soft Tissue Response to Zirconium Oxide and Titanium Healing Abutments in Vivo: A Dog Study

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Research article

Keywords: peri-implant crevicular fluid, healing abutment, ligation, zirconium oxide, titanium

Posted Date: February 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-238086/v1

Abstract

Background: This study aimed to investigate clinical characteristics and early soft tissues response to zirconium oxide (Zr) and titanium (Ti) abutments in dogs.

Methods: Eight implants-four at each hemi-mandible were inserted after bilateral mandibular third and fourth premolars and first molars extraction. Two Zr and two Ti healing abutments were connected in each unilateral mandible 8 weeks later. The ligation method was used to make peri-implant mucositis model. The twenty-four abutments were divided into four groups, Zr and Ti healing abutments with ligation (ZrL, TiL) and non-ligation (ZrN, TiN) groups. Clinical index, peri-implant crevicular fluid (PICF) and inflammatory cytokines (TNF-α and IL-1β), soft tissue responses were tested. Two-way analysis of variance was used to analyze the data.

Results: The results showed that the clinical index were similar around Zr and Ti healing abutments. PICF in ZrL and TiL groups were significantly higher than those in ZrN and TiN groups. Immunohistochemistry demonstrated inflammatory cells were non-significant differences.

Conclusion: These data indicate soft tissue responses to Zr healing abutments with peri-implant mucositis was comparable to those to Ti healing abutments in vivo, and can provide theoretical foundation for Zr's clinical application.

Background

Soft tissues serve as a protective barrier between the oral environment and the underlying peri-implant bone, and a proper integration of soft tissues significantly affects the long-term success of implant-supported restorations [1–3]. Various hazards, such as bacterial accumulation, overloading, and prosthetic manipulation, adversely affect the attachment of peri-implant soft tissues to abutments [2, 4, 5]. The biocompatibility of the transmucosal part of implants is crucial for ensuring a high quality of attachment between the mucosa and the abutment.

In the past decades, Ti has been considered as the gold standard material for dental implants and implant abutments due to its excellent biocompatibility, mechanical strength and corrosion resistance in the complex oral environment [6–8]. However, its potential defects also attract dentistry's attention. On one hand, Ti abutments can hardly meet patients' increasing aesthetic requirements on implant borne restorations – biomaterials with better optical properties are always expected. On the other hand, Ti can release sub-micrometer particles into oral cavity, which can induce inflammatory cytokines secretion in vivo [9], or even cause potential hypersensitivity towards Ti in a limited number of patients [10]. Therefore, increasing aesthetic demands have driven the fabrication of tooth-colored ceramic implant abutments [11, 12]. Yttrium oxide-stabilized zirconium oxide (Zr) has gained increasing attention for its excellent aesthetic property, mechanical properties, and ideal biocompatibility in the past decade [13, 14]. Fewer bacterial colonies were reported on Zr surfaces than on Ti surfaces in vitro studies [15–17]. Soft tissue inflammation infiltration was less common in response to Zr healing caps than Ti healing caps [18].

Anyway,an ideal implant abutment material should have the ability to maintain long-term homeostasis of the peri-implant mucosal microenvironment. The levels of TNF-α and IL-1β both in the gingival crevicular fluid (GCF) and in peri-implant crevicular fluid (PICF) were associated with periodontitis and peri-implantitis [19–22]. However, thus far, only few studies have reported peri-implant soft tissue responses to abutments with inflammation [1, 18, 23–25]. Therefore, this research detects soft tissue response of Zr abutment under peri-implant mucositis in dog model. In this study, we confirme that Zr abutment (biosafety and soft tissue response) has reached the gold standard requirement compared to Ti abutment. Combined with its unique aesthetic properties, Zr has a wide application prospects. This research fills the gap of Zr abutment (clinical evaluation in inflammatory environment) and supplies a solid theoretical foundation for its clinical application.

Methods

The study protocol was approved by the Ethics and Institutional Animal Care and Use Committees, School and Hospital of Stomatology, Wuhan University. This study conformed the Arrived guidelines.

Animals

The experiment was performed on three 1-year-old female beagle dogs weighing 12.5–15 kg. The dogs were purchased from Hubei Anlu Dog Farm (Hubei, China) and they were individually housed and maintained on a commercial diet and with water ad libitum. The health was checked and maintained daily.

Each dog underwent extraction of mandibular third and fourth premolars and the first molar (P3-M1) bilaterally. After eight weeks, eight implants-four at each hemi-mandible were inserted in each beagle dog. And twenty-four implants were used in all. After another eight weeks later, the implants were exposed and two Zr and two Ti healing abutments were respectively connected in each unilateral mandible. Cotton threads were placed at the neck of the healing abutments randomly on one side for each dog to promote plaque accumulation two weeks later. On the contralateral side, healing abutments were cautiously cleaned using Colgate dentilave every two days. The implants were divided into four groups, Zr healing abutments with ligation (ZrL) (n = 6), Ti healing abutments with ligation (TiL) (n = 6), Zr healing abutments without ligation (ZrN) (n = 6), and titanium healing abutments without ligation (TiN) (n = 6).

Implant design and surfaces

A total of 24 implants with a cylinder design, 8.0-mm length, and 3.5-mm diameter were fabricated from grade 2 unalloyed Ti rods. An inner threaded hole was made to fit the Zr and Ti healing abutments, and the implants were then ultrasonically washed first in acetone, then in ethanol, and finally in deionized water, and this process was repeated thrice. The sandblasted and acid-etched surfaces were prepared according to the previous report [26].

Surgery procedures

The schedule of the experiment is presented in Fig. 1. All surgical procedures were performed with the dogs under general anesthesia, achieved using intravenous sodium pentobarbital (3%, 1 mL/kg; Merck, Germany). Local instillation with 1- to 2-ml Primacaine adrenaline (Acteon, France) was administered for hemostasis and for reducing the postoperative pain. Streptomycin and penicillin were postoperatively administered for 4 days.

After 2 weeks of adaption feeding, the mandibular third and fourth premolars and first molar (P3-M1) were bilaterally extracted. After eight weeks [27], a full thickness mucoperiosteal flap was evaluated and eight implants-four at each hemi-mandible, were inserted in each beagle dog. Twenty-four implants were used in all. Implants were placed with their coronal margins at the level of the alveolar bone crest (Fig. 2a). Cover screws were installed and the flaps were sutured.

After another eight weeks of healing, the implants were exposed by circular scalpel. Then two Zr and two Ti healing abutments were connected randomly in each unilateral mandible (Fig. 2b). Oral hygiene maintenance was initiated. After two weeks, PI (plaque index), GI (Gingival index), PD (probing depth) were recorded and PICF was collected as baseline (day 0). Then cotton threads were placed at the neck of the healing abutments randomly on one side for each dog to promote plaque accumulation [28, 29]. On the contralateral side, healing abutments were cautiously cleaned using Colgate dentilave every two days. The implants were divided into four groups, Zr healing abutments with ligation (ZrL) (n = 6), Ti healing abutments with ligation (TiL) (n = 6), Zr healing abutments without ligation (ZrN) (n = 6), and titanium healing abutments without ligation (TiN) (n = 6). Twenty-eight days later, PI, GI, PD were recorded and PICF was collected.

Clinical measurements

Clinical measurements were obtained at six sites around the healing abutments on day 0 and 28, respectively. PI [30] and GI [31] were initially scored, followed by PICF sampling, and finally the PD was recorded. All clinical examinations were performed by one examiner.

PICF sampling and processing

Supragingival plaque attached to healing abutments was gently cleaned using wet cotton balls. The implants were then isolated using cotton rolls and gently air-dried, and PICF was then collected using 8 × 2-mm filter paper strips (Whatman 3#, United States). The paper strips were inserted into the mesial- and distal-buccal sulcus of the healing abutment until slight resistance was felt and then remained there for 30 seconds. Strips contaminated by bleeding or by exudates were discarded. The volume of PICF was calculated by weighing by subtraction using a precise electronic balance before and after PICF collections. The strips were stored at − 70ºC until further analysis.

Enzyme-linked immunosorbent assay (ELISA) analysis

PICF samples were thawed and eluted according to Griffiths's method [32]. Two ELISA kits (CATA00, DY3747, R&D, USA) were used to determine the levels of TNF-α and IL-1β. All procedures were performed in accordance with the manufacturer's instructions. Absorbance at 450 nm was measured using an ELISA

reader (BioTeK Instruments, Inc., Winooski, VT, USA). The levels of TNF-α and IL-1β were estimated using the assay standard criteria. All tests were performed in duplicates.

Histopathological analysis

The dogs were sacrificed by administering an overdose of pentobarbital sodium. The hemi-mandibles were removed and fixed in 10% buffered paraformaldehyde (pH 7.2) for 48 h. The specimens were carefully dissected into pieces with one implant, and decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution at 4°C until a syringe needle could punch encountered no resistance. The implants were carefully removed, and all specimens were embedded in paraffin blocks. Specimens were sectioned along their longitudinal axis at 5 μm and stained with hematoxylin and eosin (HE) for histological examination. The slices were observed under a conventional light microscope (Olympus BHS-313, Tokyo) at ×20 magnification.

To identify the early inflammatory infiltration of soft tissues around Zr and Ti healing abutments, the avidin–biotin–peroxidase method was used for immunohistochemistry of 5-μm-thick sections. After deparaffinization and rehydration, sections were submitted for antigen retrieval using citrate buffer (10 mM, pH 6.0 using a pressure cooker for 5 min, at 120°C). Endogenous peroxidase was blocked using 3% H_2O_2 for 10 min at 37°C. Slides were preincubated with a protein block solution [2% skim milk, 0.05% Triton X-100, and phosphate-buffered saline (PBS)] for 30 min at room temperature to prevent nonspecific binding. Immunostaining was performed by incubating the primary monoclonal antibody against IL-1β (1:100; NOVUS, USA) and TNF-α (1:50; R&D, USA) in a humid chamber at 4°C overnight. The reactions were developed using diaminobenzidine, and immunostained sections were counterstained with hematoxylin. For control experiments, the primary antibodies were replaced with PBS. Sections were observed under a conventional microscope (Olympus BHS-313, Japan) and photographed using a calibrated digital camera (Olympus C-35AD-4) at ×20 and ×40 magnifications.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Differences among Zr and Ti implant healing abutments with or without ligation were evaluated using two-way analysis of variance. $P < 0.05$ was considered statistically significant. All statistical analysis was performed using a statistical package (SPSS® version 19.0; SPSS, Inc., Chicago, IL, USA).

Results

Clinical findings

Visual assessment confirmed the presence of peri-implant mucositis (Fig. 3). Soft tissues around the Zr and Ti healing abutments were red and swollen at the fourth week after ligation. No differences were observed in PI and PD among the four groups on days 0 and 28 and in GI of tissues around the Zr and Ti healing abutments on day 0. However, GI of tissues around the ZrL and TiL healing abutments was significantly higher than that of those around the ZrN and TiN abutments on day 28 (Table 1).

Table 1
Clinical parameters of Zr and Ti healing abutments with or without ligation

		Day 0			Day 28		
		L	N	P	L	N	P
PI	Zr	1.2 ± 0.98	1.8 ± 1.17	0.282	2.3 ± 0.52	2.5 ± 0.55	0.534
	Ti	2.2 ± 0.98	2.3 ± 1.03	0.785	2.7 ± 0.52	3.0 ± 0.00	0.220
	P	0.113	0.417		0.220	0.072	
GI	Zr	0.3 ± 0.5	1.0 ± 0.6	0.051	1.5 ± 0.6	0.5 ± 0.5	0.002**
	Ti	0.3 ± 0.5	0.5 ± 0.5	0.609	1.5 ± 0.6	0.8 ± 0.4	0.011*
	P	1.000	0.135		1.000	0.431	
PD	Zr	3.13 ± 0.43	2.92 ± 0.38	0.622	3.45 ± 0.64	3.08 ± 0.66	0.108
(mm)	Ti	2.75 ± 0.69	3.58 ± 1.20	0.068	3.75 ± 0.96	3.3 ± 0.63	0.644
	P	0.386	0.139		0.490	0.617	
(PI, plaque index; GI, gingival index; PD, probing depth; L, ligation; N, non-ligation							
*P < 0.05 There was difference; ** P < 0.01 There was significant difference)							

Quantification of TNF-α and IL-1β in PICF

The volumes of PICF were similar around the Zr and Ti healing abutments on day 0 and significantly higher in tissues around the ZrL and TiL abutments than in those around the ZrN and TiN abutments on day 28 (Table 2).

Table 2
Comparison of PICF volumes around Zr and Ti healing abutments with or without ligation (mg)

		Day 0			Day 28		
		L	N	P	L	N	P
PICF	Zr	1.45 ± 0.32	1.45 ± 0.32	1.00	2.39 ± 0.51	1.12 ± 0.22	0.002**
	Ti	1.37 ± 0.16	1.58 ± 0.73	0.566	1.83 ± 0.29	1.08 ± 0.22	0.025*
	P	0.828	0.718		0.073	0.886	
(L, ligation; N, non-ligation; *P < 0.05 There was difference; **P < 0.01 There was significant difference)							

The levels of TNF-α and IL-1β in PICF around the Zr and Ti healing abutments, with or without ligation, are presented in Table 3. The levels were similar around the Zr and Ti healing abutments on day 0 and higher around the ZrL and TiL abutments than around the ZrN and TiN abutments on day 28. However,

only the levels of TNF-α were significantly different between the ZrL and ZrN groups on day 28 (P = 0.022).

Table 3
TNF-α and IL-1β quantification in PICF around Zr and Ti healing abutments with or without ligation (pg/ml)

		Day 0			Day 28		
		L	N	P	L	N	P
TNF-α	Zr	4.25 ± 1.50	3.66 ± 1.40	0.550	7.85 ± 3.98	4.09 ± 1.64	0.022*
	Ti	3.32 ± 1.03	4.67 ± 2.36	0.468	6.95 ± 2.27	5.48 ± 1.95	0.343
	P	0.535	0.482		0.556	0.369	
IL-1β	Zr	1.25 ± 0.22	1.32 ± 0.26	0.642	1.52 ± 0.28	1.12 ± 0.34	0.393
	Ti	1.11 ± 0.18	1.23 ± 0.16	0.426	1.67 ± 0.35	1.00 ± 0.20	0.173
	P	0.349	0.542		0.758	0.809	
(L, ligation; N, non-ligation; *P < 0.05 There was difference; **P < 0.01 There was significant difference)							

Histological observations and histomorphometrical measurements

In the sections stained with HE, the collagen fiber bundles were oriented parallel to the surfaces of the ZrN and TiN healing abutments. The collagen fiber bundles were denser around the Zr healing abutments than around the Ti healing abutments, whereas fibroblasts were fewer around the Zr healing abutments than around the Ti healing abutments. Moreover, the epithelium proximate to the TiN abutments was deeper stained than that proximate to the ZrN abutments. The early soft tissue responses to the ZrN and TiN healing abutments were similar. The collagen fiber bundles adjacent to the abutments were disordered and the number of fibroblasts was increased and deeper stained in the healing abutments with ligation than in those without ligation (Fig. 4).

Immunohistochemistry staining for TNF-α and IL-1β revealed the expression of positive inflammatory cells at the basement membrane zone, soft tissues adjacent to healing abutments, and endothelial cells of vessels in the vicinity (Fig. 5). The inflammatory infiltrations were more obvious in tissues adjacent to the ZrL and TiL healing abutments than in those adjacent to the ZrN and TiN healing abutments. The mean amounts of positive inflammatory cells with TNF-α were 140.1 ± 25.4 and 160.1 ± 30.3 in the tissues adjacent to the ZrL and TiL healing abutments, respectively (P = 0.353), and 113.6 ± 11.2 and 114.8 ± 41.8 in those adjacent to the ZrN and TiN healing abutments, respectively (P = 0.963). Further, the mean amounts of positive inflammatory cells with IL-1β were 160.4 ± 45.1 and 214.9 ± 21.0 in the soft tissues adjacent to the ZrL and TiL healing abutments, respectively (P = 0.123), and 115.5 ± 23.1 and 134.6 ± 54.7 in those adjacent to the ZrN and TiN healing abutments, respectively (P = 0.563).

Discussion

The properties of abutment materials can influence the quality of mucosal attachment formation [2]. Zr abutments were introduced to facilitate the esthetic implantation treatment of the maxillary anterior teeth, particularly in cases of patients with thin mucosa [33]. However, there are only limited data on the soft tissue response to Zr, particularly in comparison with the response to Ti under peri-implantitis conditions. This self-control study provides valid data support to compare the expression of proinflammatory cytokines in PICF and the inflammatory infiltration of soft tissues around implant abutments fabricated from Zr and Ti with and without ligation. No evidence of significant differences between these two types of biomaterials was found.

The results of this study showed that peri-implant mucosal inflammation occurred around the ZrL and TiL healing abutments and soft tissues around these abutments were red and swollen. GI was significant higher of specimens with the ZrL and TiL abutments than those with the ZrN and TiN abutments on day 28. However, no significant differences were found in PD, indicating that peri-implant mucositis without bone loss was the type of inflammation. This result is consistent with that of a previous study in dogs conducted by Albouy et al., the findings of which indicated that peri-implant bone resorption occurred at 12 weeks after ligation [34].

Investigations of the biochemical parameters in the gingival sulcus or PICF have become increasingly popular because it is possible to monitor the health status of gingiva and peri-implant mucosa [19, 35]. These biochemical methods can provide with an early diagnosis and potential application in disease prevention. TNF-α and IL-1β are primarily secreted by monocytes and macrophages and are potent multifunctional cytokines in abundant signal transduction processes during inflammation by acting as proinflammatory proteins. Therefore, the levels of TNF-α and IL-1β in PICF were analyzed, in addition to the clinical parameters.

The volumes of PICF were significantly increased in the tissues around the ZrL and TiL abutments than in those around the ZrN and TiN abutments on day 28, which was related to the occurrence of peri-implant mucositis. These results are in good accordance with previous studies, which demonstrated a significant increase in the volume of PICF after plaque accumulation [19, 35]. However, after oral hygiene behaviors were resumed, the volume decreased. These findings demonstrated that oral hygiene reduces peri-implant mucosal inflammation [19]. These data suggested that an increased volume of PICF could be a useful marker of the early inflammation of peri-implant soft tissues.

In this study, the levels of TNF-α and IL-1β in PICF were increased in the tissues around the ZrL and TiL abutments on day 28. Elevated levels of TNF-α and IL-1β in PICF have been found to be associated with peri-implantitis and peri-implant mucositis [19, 20]. Nevertheless, no significant differences in the levels of TNF-α and IL-1β were observed in this study, which may be related to the short observation period. The findings of TNF-α and IL-1β in PICF obtained in the present study were in good agreement with the clinical findings, which indicated that TNF-α and IL-1β could be useful markers for assessing the peri-implant health status.

According to morphological criteria on the amount of inflammation that reflects soft tissue health status, no differences were observed on the soft tissue inflammatory infiltration around the Zr and Ti healing abutments. However, fewer inflammatory cells were observed around the Zr healing abutments than around the Ti healing abutments. Similar results were also observed in a recent study conducted by Brakel et al., which reported no difference in the inflammation grading scale score in peri-implant mucosa adjacent to the Zr and Ti abutments [24]. A canine study conducted by Welander et al. reported lesser inflammatory infiltration in the epithelium of peri-implant mucosa around the Zr abutments than in that around the Ti implants [1]. In a human histological study, Degidi et al. reported significant elevations in the proinflammatory infiltrates (lymphocytes, plasma cells, and histiocytes) as well as an increased expression of vascular endothelial growth factor and nitric oxide synthase isoforms 1 and 3 in the tissues adjacent to the Ti healing abutments than in those around the Zr healing abutments after a 6-month healing phase [18].

The immunohistochemistry assays in this study revealed the expression of inflammatory cells at the basement membrane zone, in the soft tissues adjacent to healing abutments, and in the small endothelial cells of vessels in the vicinity. These inflammatory cells may be related to the Langerhans cells in the basal layer and vascular endothelial cells. Langerhans cells are lymphocyte antigen-presenting cells that play an important role during the early immune response of periodontitis or gingivitis [36]. Vascular endothelial cells are involved in the inflammatory process via the release of proinflammatory cytokines.

Whether this observation was attributable to the favorable attachment properties for the surrounding connective tissues and the epithelium was not conclusively established. A reduction in the inflammatory reactions may not merely be an expression of better insulation through the soft tissue but may also due to the proven lesser accumulation of bacteria on ceramic surfaces [16, 17].

Conclusion

Within the limitation of this study, the findings indicate that soft tissue responses to Zr healing abutments with peri-implant mucositis was comparable to those to Ti healing abutments in vivo, and can provide theoretical foundation for Zr’s clinical application.

Abbreviations

EDTA
Ethylenediaminetetraacetic acid; ELISA:Enzyme-linked immunosorbent assay; GCF:Gingival crevicular fluid; GI:Gingival index; HE:Hematoxylin and eosin; L:Ligation; N:Non-ligation; PBS:Phosphate-buffered saline; PD:Probing depth; PI:Plaque index; PICF:Peri-implant crevicular fluid; SD:Standard deviation; Ti:Titanium; TiL:Titanium ligation; TiN:Titanium non-igation; Zr:Zirconium oxide; ZrL:Zirconium ligation; ZrN:Zirconium non-ligation.

Declarations

Acknowledgments

We acknowledged the Nobel Biocare Company for providing all-ceramic healing abutments.

Authors' contributions

Study design: YW, HX. Animal surgeries and data analysis: MW, SZ, LC, HZ. Drafted the manuscript: MW, SZ, HX. All authors have read and approved the final manuscript.

Funding

The work was funded by the National Natural Science Foundation of China (81500866) and Lanzhou Talent Innovation and Entrepreneurship Support Project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

The data of this study are available from the corresponding author, HX, upon reasonable request.

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were in accordance with the ethical standards of the institutional and national research committee. This experimental protocol was approved by the Ethics and Institutional Animal Care and Use Committees, School and Hospital of Stomatology, Wuhan University. This study conformed the Arrive guidelines.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

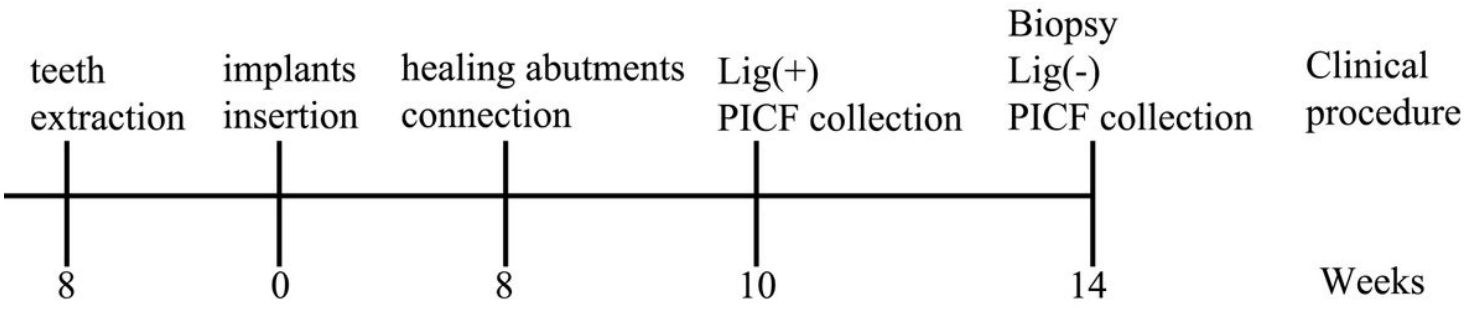

Figure 1

Outline of the experiment.

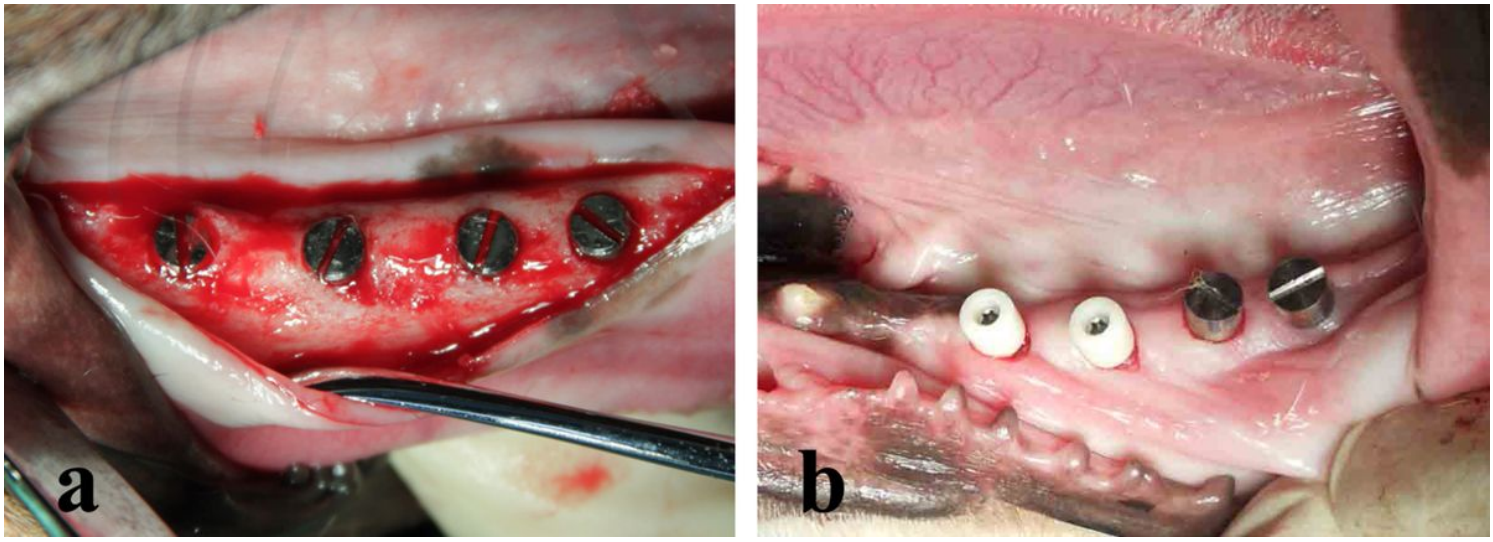

Figure 2

(a) Occlusal view of the implant insertion in unilateral mandibular edentulous region; (b) Occlusal view of Zr and Ti healing abutments connection.

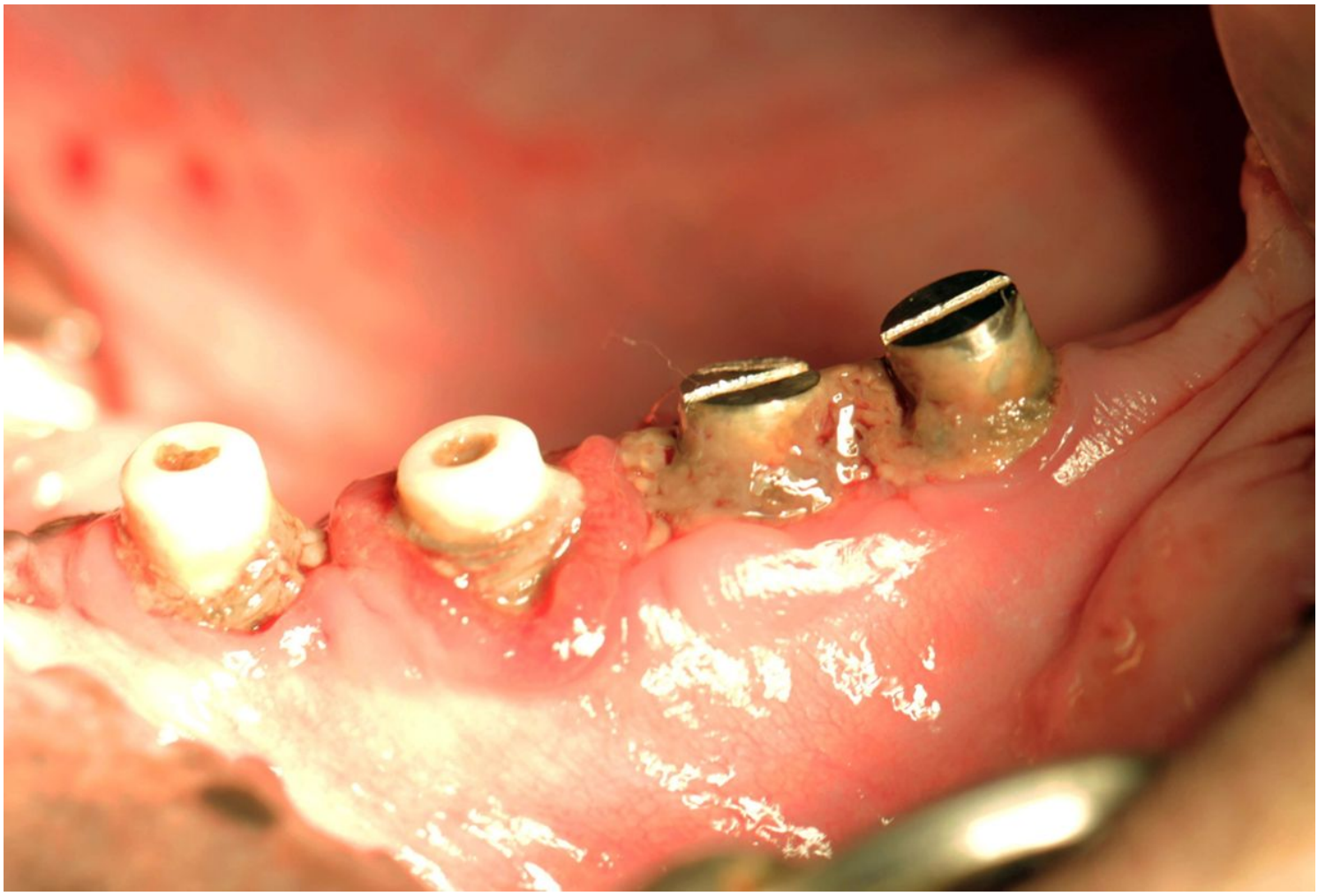

Figure 3

The occurrence of peri-implant mucositis at day 28.

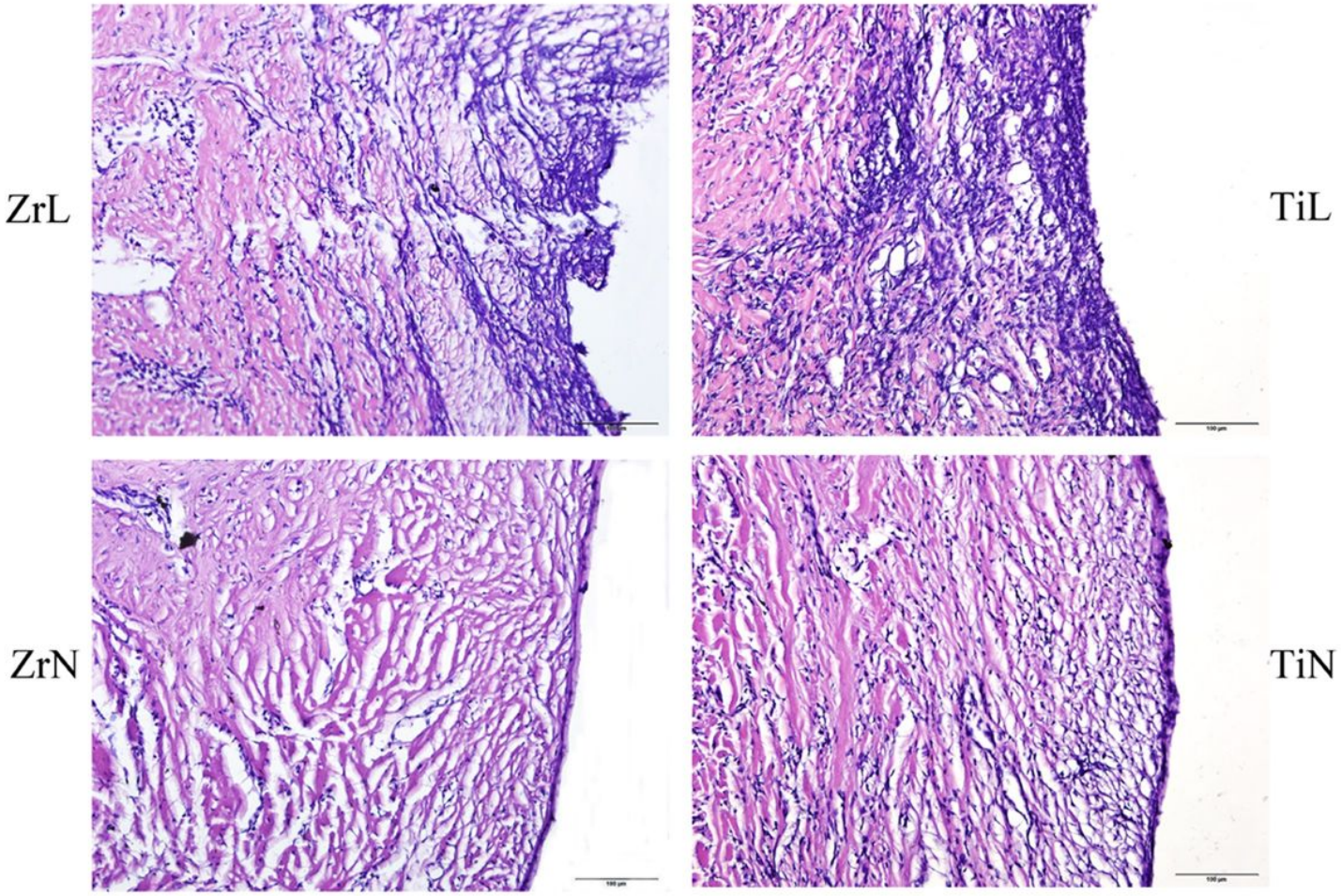

Figure 4

HE stain of soft tissues around Zr and Ti healing abutments with or without ligation (the bar =100 μm)

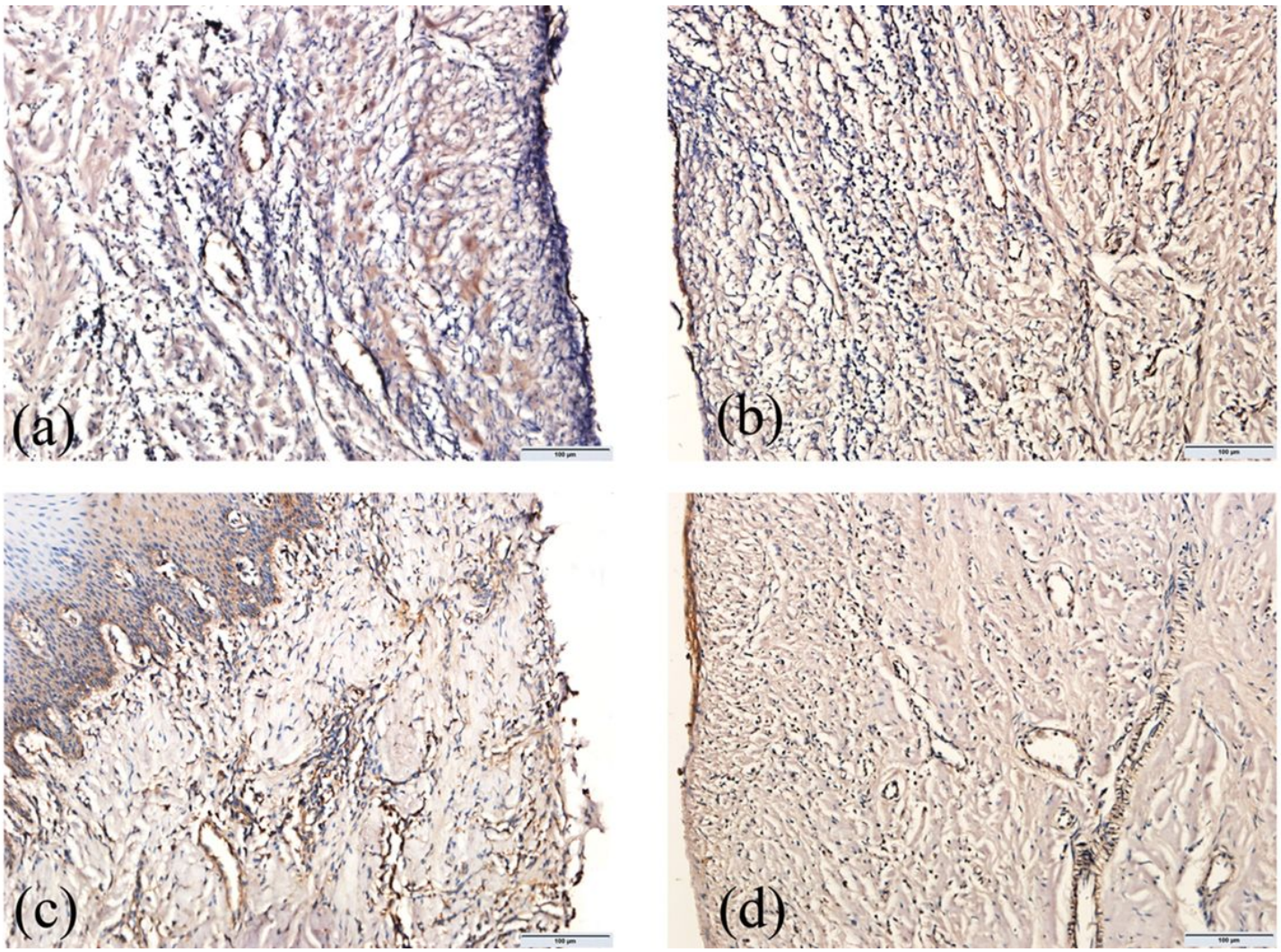

Figure 5

Immunohistochemical observation identified as TNF-α and IL-1β. (a) TNF-α labeled with ligation; (b) TNF-α labeled without ligation; (c) IL-1β labeled with ligation; (d) IL-1β labeled without ligation. (the bar =100 μm)

Supplementary Files

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