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Soft tissues around long-term platform switching implant restorations: a histological human evaluation. Preliminary results

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Abstract

Background: Switching platform restorations seems to reduce the peri-implant bone resorption and to preserve the peri-implant soft tissues.

Aim: The aim of the present human study was to compare histologically the peri-implant soft tissue in switching and traditional platform implants 4 years after restoration.

Materials and Methods: Forty-eight months after implant restoration, 37 peri-implant soft tissue samples from 14 patients were harvested from traditionally restored implants (control group) and from three different platforms mismatching 0.25–0.85 mm (test groups). At the harvesting time, all sites were clinically healthy. Samples were processed to evaluate the inflammatory infiltrate area [inflamed connective tissue (ICT)], the microvascular density (MVD) and the collagen content (AA%).

Results: At the analyses, no significant differences were found between groups in terms of ICT, MVD and AA% ($p > 0.05$). In all groups, most samples with a well-preserved junctional epithelium showed a small and localized inflammatory infiltrated associated with not-well-oriented collagen fibres and an increased MVD.

Conclusions: Forty-eight months after restoration, switching and traditional platform implants had similar histological peri-implant soft tissue features, despite different bone level changes detected radiographically and published in a previous parent study. The present study seems to confirm platform switching as a safe prosthetic concept leading to better maintenance of peri-implant bone levels. However, further histological studies are required to longitudinally confirm the present data.

Key words: dental implants; histologic analysis; immunohistochemistry; peri-implant soft tissues; platform switching

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One year following implant restoration, dental implants restored with prosthetic components of matching diameter have crestal bone re-modelling around the coronal part of the implant and about 1.5–2 mm of vertical bone loss (Hermann et al. 1997, Brägger et al. 1998).

Conflict of interest and source of funding statement

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This biological width re-establishment may occur as result of micromovements at the implant–abutment interface (IAI) (Hermann et al. 2001), or bacterial migration and colonization of the micro-gap on a screw-retained abutment that induce a localized chronic inflammation (Broggini et al. 2006).

Switching platform restorations present a smaller diameter restorative component that keep the IAI inwardly and away from the outer edge of the implant. This seems to reduce the post-restorative crestal bone re-modelling (Lazzara & Porter 2006, Vigolo & Givani 2009) and to provide a preservation of papilla and peri-implant soft tissues (Canullo & Rasperini 2007). In a preliminary report, Canullo et al. (2009) demonstrated that inter-proximal papillae and soft tissue buccal margin around matching diameter IAI had significantly higher apical migration than switching platform abutment.

Data from histological human studies seem to confirm clinical results and give a possible reason for the preservation of bone crest around dental implants (Degidi et al. 2004, 2008, Luongo et al. 2008).

In a case report, Luongo et al. (2008) evaluated histologically the soft tissue around a platform-switched implant removed for prosthetic reasons 2 months after loading. Authors found that the lymphocytes and plasma cells infiltrated only a small area (0.35 mm^2) of the connective tissue coronal to the IAI. In this study, hard tissue was also analysed: bone loss was limited and no infraosseous defects, Howship lacunae or osteoclasts were found on the bone crest (Luongo et al. 2008). Similar results were found in a study (Degidi et al. 2008) where soft and mineralized tissue around an implant with switching platform design were harvested and analysed 1 month after implant loading. Authors reported a collar of dense, fibrous connective tissue with few inflammatory cells at level of the implant shoulder and no resorption of the coronal bone around the implant. Also, Degidi et al. (2004) observed newly formed bone and an absence of osteoclastic activity on peri-implant bone crest after a 6-month restoration period with a mismatching abutment. Based on these clinical and histological data of three case reports, different hypotheses were purposed to explain benefits of mismatching diameter implant abutments including: placement of the IAI inwards and away from the bone tissue and the reduction of the inflammatory effect within the surrounding soft tissue and crestal bone (Lazzara & Porter 2006), the establishment of a wider and more resistant area of connective tissue around the IAI (Becker et al. 2007, Degidi et al. 2008) and the better distribution of loading stress at the IAI (Canullo et al. 2010).

The previously reported human histological studies seem to confirm the hypothesis of the separation of IAI and bone tissue on maintaining peri-implant mineralized tissue. However, they only evaluated short-term response of the connective tissue around switching platform abutment, did not compare it with tissues harvested around matching diameter abutment and did not investigate the histological features to support each hypothesis.

The present histologic study was performed to evaluate (i) the presence and distribution of inflammatory cells, tissue vascularization as well as the content of collagen fibres of peri-implant soft tissues around switching and non-switching platform restored implants at 4 years after prosthetic rehabilitation and (ii) the correlation between histologic parameters and clinical parameters of local gingival inflammation.

Materials and Methods

This study is the continuation of a recently published prospective longitudinal randomized control study (Canullo et al. 2010). The detailed protocol and the clinical and radiographic results can be found in the parent study (Canullo et al. 2010).

Briefly, patients scheduled for multiple implant-supported restorations were selected (Fig. 1). All patients were in general good health. The subjects were informed about the study protocol and were required to sign a consent form. The study was performed following the principles outlined in the Declaration of Helsinki on experimentation involving human subjects. All procedures and materials in the present study were approved by the ethical committee at University ''La Sapienza'', Rome, Italy. All patients provided informed consent.

Exclusion criteria were: natural teeth adjacent to surgical area affected by untreated periodontal and endodontic infections, absence of opposing occlusion, parafunctional habits, severe maxillomandibular space discrepancies, patients with a full-mouth plaque score and a full-mouth bleeding score $>25\%$, smokers who smoked more than 10 cigarettes per day, patients with uncontrolled diabetes, women who were pregnant or lactating, any drug use (included biphosphonates) or alcohol abuse.

In addition, sites with acute infection, sites with <7 mm buccolingual width of bone crest, and not healed sites or sites with inter-proximal or buccal bone defects were also excluded.

Each implant site was randomly assigned to the placement of one of the following implant diameters: 3.8 mm (control group), 4.3 mm (test $group_1$), 4.8 mm (test $group_2$) and 5.5 mm (test $group_3$). In order to reduce the chance of unfavourable splits between test and control groups in terms of key prognostic factors, the randomization process took into account the following variables: patient's gender, age, presence of adjacent teeth, distal extension sites and site location in the dental arch. Assignment was performed using a sealed envelope.

According to the randomization, one to four 13 mm implants (Global, Sweden and Martina, Padua, Italy), were inserted in a standardized way in the posterior maxilla: once the implant site was prepared to receive a 3.8-mm-diameter implant, surgeons' assistants were asked to open the sealed envelope containing the randomization. If required, the implant site was then enlarged according to randomization, to insert a wider than 3.8-mm-diameter implant.

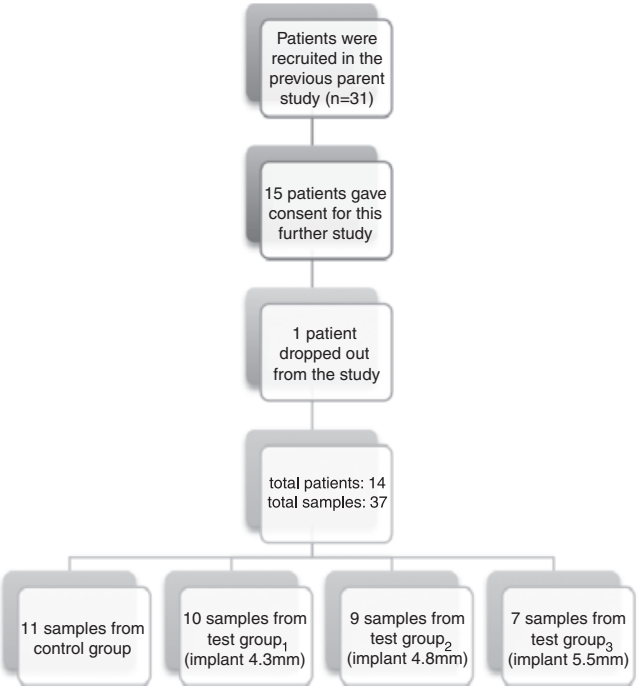

Fig. 1. Flowchart of the subjects and the samples throughout the study.

At the end of the implant site preparation, the residual buccal and lingual bone wall always resulted at least 1 mm. A minimal implant-to-implant and implant-to-tooth distance of 2 mm was always maintained.

The root-shaped implant used in this study presented a 0.3 mm in height smooth-surfaced collar, microthreads in the coronal portion and a sand-blasted and acid-etched surface in the whole length of the body.

All implants were inserted with the platform at the bone level.

After 3 months of healing, the second-stage procedure was performed. For restoration, a 3.8 abutment was used in both test and control implants. In the test groups, this restoration resulted in a mismatching of 0.25–0.85 mm of implant–abutment diameters. Splinted implant definitive restoration was adopted in case more than one implant were placed in the same edentulous region.

All patients were followed up every 6 months for 48 months. At the last follow-up, five patients dropped out.

Study population

Four years after implant insertion, all 31 subjects who concluded the radiographic follow-up were contacted and requested to come to the dental centres for the peri-implant soft tissue biopsy. In subjects who answered the request, a marginal portion of peri-implant soft tissue biopsy was taken from the palatal aspect of all implants.

After restoration removal using probe as a reference, a surgical blade was inserted from the soft tissue margin till the bone touching the implant platform. Paracrestal releasing incision following the bone wall was performed to disconnect the soft tissue peduncle (Fig. 2).

Clinical measurements

At the time of tissue harvesting, the following clinical parameters were taken from the same examiner (L. C.) using a periodontal probe (15 mm, PCP-UNC 15, Hu-Friedy, Chicago, IL, USA): bleeding on probing (BOP), probing depth (PD) on implants and adjacent teeth, as reported in a recently published parent study (Canullo et al. 2010).

Specimen processing

Immediately after surgery, the biopsied specimens were immersion fixed in 10% formalin/0.1 M phosphate-buffered saline (pH 7.4) 24 h at room temperature, then routinely dehydrated in increasing concentrations of ethanol (from 50% to 100%), xylol for 12 h and then paraffin embedded. Serial 4–5 μm buccal–lingual sections were obtained, mounted on 3-amino-propyl-triethoxy-silane-coated slides and then hydrated in a decreasing concentration of xylol and ethanol (from 100% to 70%) and after all immersed in distilled water. Sections were stained with haematoxylin and eosin to evaluate the tissue morphology (four sections for site) and with Sirius red (four randomly selected sections for site) to evaluate the collagen content in the connective compartment.

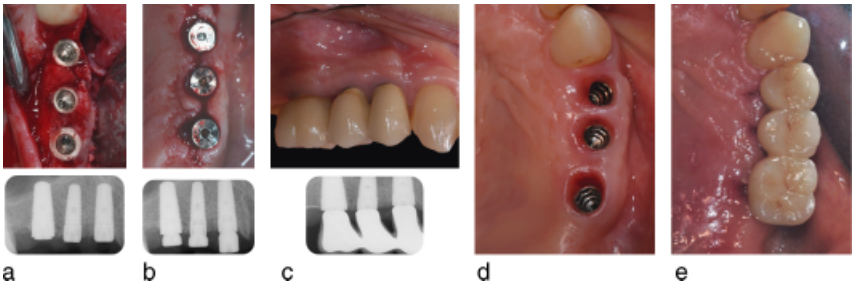

Fig. 2. Photographs and radiographs of a clinical case. (a) Implant insertion at the crestal level (time 0); (b) second surgery (3 months); (c) soft tissue response (36 months); (d) soft tissue before biopsy after crown and abutment removal (48 months); (e) soft tissue healing 12 days after biopsy.

Immunohistochemistry

Immunostaining for CD31 was performed on four sections (two marked and two controls for site) to highlight blood vessels. Sections of human tonsils were used to determine the optimal antibody dilution. Immunohistochemical staining was performed using the polymeric HRP method. For antigen retrieval, slides immersed in a citrate buffer pH 6.0 solution were maintained in a thermostatic bath at 98°C for 30 min. Slides were incubated for 10 min with H_2O_2 at 3% to block endogenous peroxidase activity. Slides were then incubated with primary antibody (Monoclonal Mouse Antibody to Human Endothelial Cell, CD31) (DBS, Pleasanton, CA, USA) for 30 min at room temperature. Primary antibody Enhancer (Thermo Fisher Scientific, Fremont, CA, USA) was applied and incubated for 10 min at room temperature. Slides were treated with HRP Polymer (Thermo Fisher Scientific) and incubated for 15 min. After incubation with diaminobenzidine tetrahydrochloride (DAB) as a substrate/chromogen (''DAB plus chromogen-substrate pack'', Thermo Fisher Scientific) for 5 min, the specimens were counterstained with Mayer's haematoxylin and coverslipped. For negative control, two sections were incubated in serum.

Histomorphometrical analysis

All the histological sections were viewed and photographed using a Nikon light microscope (Eclipse E600) equipped with a calibrated digital camera (DXM1200, Nikon, Tokyo, Japan).

On haematoxylin and eosin sections, the area of connective tissue disrupted and infiltrated with inflammatory cells was recognized and outlined by the same expert operator (G. P.). This area of inflamed connective tissue (ICT, mm^2) was measured at a total magnification of $\times$ 100 on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

For the immunohistochemical analysis, percentage of microvessels in the connective tissue [microvascular density (MVD)] was computed using a point-counting technique procedure according to the Delesse formula: $V_V \times P_P$. An area of connective tissue (0.5 mm^2) close to the apical site of the junctional epithelium (JE) and excluding the inflammatory area (ICT) was identified. A lattice comprising 100 test points was superimposed to this area and photographed at a total microscopic magnification of $\times$ 100. The tissue underlying each grid intersection was recorded as vessels. The number of hits containing vessels was divided by the total number of intersection fall on the overall connective tissue and thus expressed in percentage values.

All of the Sirius red-stained sections were analysed by light microscopy and polarized light, and the images were captured at a total magnification of × 200 and digitized using an image analysis system with specific software (Bio Image Analyzer, ICH, Milano, Italy), as described elsewhere (Gagliano et al. 2005). Connective tissue was isolated from the whole gingival section and tissue collagen content was expressed by an area fraction index (AA%), indicating the ratio of the mean Sirius red-stained surface to the connective tissue area of the section.

Statistics

In the statistical analysis, each implant was considered as the statistical unit.

To evaluate the maximal extension of the inflammatory area, the maximum area of the ICT from the four slices per sample was reported. To evaluate the modifications in the connective tissue surrounding (3D) the ICT, for MVD and AA% determination, the overall area of all sections was analysed. For each implant site, the MVD and AA% values represent the mean of the determinations.

Mean and standard deviation were calculated for all the observed parameters (ICT, MVD, AA%) separately for control and test groups. Comparisons of these data between groups were computed by a Kruskal–Wallis non-parametric test, using a statistical software (Kyplot v. 2.0). Additionally, the histological variables were correlated with clinical variables as BOP and PD, by using the coefficients of correlation (Pearson's r). Coefficients of correlation were calculated with paired data series from each patient. A level of significance of 5% ($p \leqslant 0.05$) was used for all analyses.

Results

Fifteen patients gave consent to the study and one patient dropped out from the study due to circumstances unrelated to the study. A total of 37 sites from 14 patients with a mean age of 59 years were included in the study. Samples were divided in the following four groups: 11 for the control group, 10 for the test group$_1$, nine for the test group$_2$ and seven for the test group$_3$ (Fig. 1). At the implant site for each group, the mean PD was: control group: 3 mm, test group$_1$: 2.4 mm, test group$_2$: 2.5 mm, test group$_3$: 3 mm; and the BOP was: control group: 0, test group$_1$: 0.33, test group$_2$: 0.4, test group$_3$: 0.8 (Table 1). Table 1 shows inflammatory clinical parameters (BOP, mesial/distal PD) evaluated immediately before tissue harvesting. The distribution of the 37 samples over the 14 patients is indicated in Table 2. Figure 2 reports the photographs and radiographs of a clinical case.

Table 1. Mean values of bleeding on probing (BOP) and probing depth (PD) are indicated for each group

Group	BOP (SD)	PD (mm) (SD)
Control: 3.8 mm ($n = 11$)	0	3 (0.0)
1: 4.3 mm ($n = 10$)	0.3 (0.5)	2.4 (0.6)
2: 4.8 mm ($n = 9$)	0.4 (0.5)	2.5 (0.7)
3: 5.5 mm ($n = 7$)	0.8 (0.9)	3 (0.0)

SD, standard deviation.

Table 2. Distribution of the 37 samples over the 14 patients

Patient number – initials	*N*	Sites (implant diameter)
01 – C. C.	3	1.3 (ID: 4.8); 1.5 (ID: 4.8); 1.6 (ID: 4.8)
02 – B. M.	2	2.5 (ID: 4.3); 2.6 (ID: 4.8)
03 – S. E.	3	1.6 (ID: 4.8); 1.7 (ID: 4.8); 1.4 (ID: 4.3)
04 – Z. M.	3	2.5 (ID: 3.8); 1.5 (ID: 4.3); 1.6 (ID: 4.3)
05 – P. L.	2	1.5 (ID: 5.5); 1.5 (ID: 5.5)
06 – M. O.	3	1.4 (ID: 3.8); 1.5 (ID: 3.8); 1.6 (ID: 3.8)
07 – M. V.	3	2.4 (ID: 4.3); 2.5 (ID: 4.8); 2.6 (ID: 4.8)
08 – B. R.	2	1.6 (ID: 5.5); 1.7 (ID: 5.5)
09 – L. M.	3	2.5 (ID: 4.3); 2.6 (ID: 3.8); 2.7 (ID: 5.5)
10 – M. B.	3	1.3 (ID: 3.8); 1.4 (ID: 4.3); 1.6 (ID: 5.5)
11 – A. A.	2	2.6 (ID: 4.3); 2.7 (ID: 4.3)
12 – M. T.	3	2.4 (ID: 4.3); 2.5 (ID: 3.8); 2.6 (ID: 3.8)
13 – R. R.	2	1.5 (ID: 3.8); 1.6 (ID: 4.8)
14 – G. G.	3	1.4 (ID: 3.8); 1.5 (ID: 3.8); 1.6 (ID: 5.5)
Total	37	ID 3.8 = 11; ID 4.3 = 10; ID 4.8 = 9; ID 5.5 = 7

N, number; ID, implant diameter.

At histological evaluation, all samples presented a well-organized connective tissue underneath the oral epithelium. The method used to harvest the biopsies did not assure the integrity of the JE, and thus information on JE was not retrieved. Inflammatory cells were mainly localized underlying the JE, and in the connective tissue only few scattered cells were observed. For this reason, the area of ICT was calculated only in samples where JE was preserved. A great variability of the morphological features between samples of the same group was observed in terms of size of inflammatory infiltrated, MVD and collagen content.

Inflammatory infiltrate

In most samples of all groups, a small concentrated population of lymphocytes was mainly localized in the connective tissue close to the JE (Fig. 3a–c). The remaining peri-implant connective tissue presented few scattered lymphocytes and macrophages.

In 26 sites, the JE was well preserved and it was possible to calculate the maximum ICT area. Table 3 shows the mean area occupied by the ICT in each group and no significant differences were found between groups ($\chi^2 = 4.72$, $p = 0.19$). However, large differences in the ICT size within each group were detected.

No significant correlations between the ICT and the clinical variables (BOP and PD) were found (Table 4).

MVD

For the evaluation of MVD, only morphologic structures with a lumen surrounded by CD31 antigen were considered blood microvessels (Fig. 4a and b).

As showed in Table 3, the differences in MVD between groups were not statistically significant ($\chi^2 = 5.67$, $p = 0.13$).

All samples showed microvessels mainly distributed underneath the oral epithelium and the vascular density decreased in the deep connective tissue.

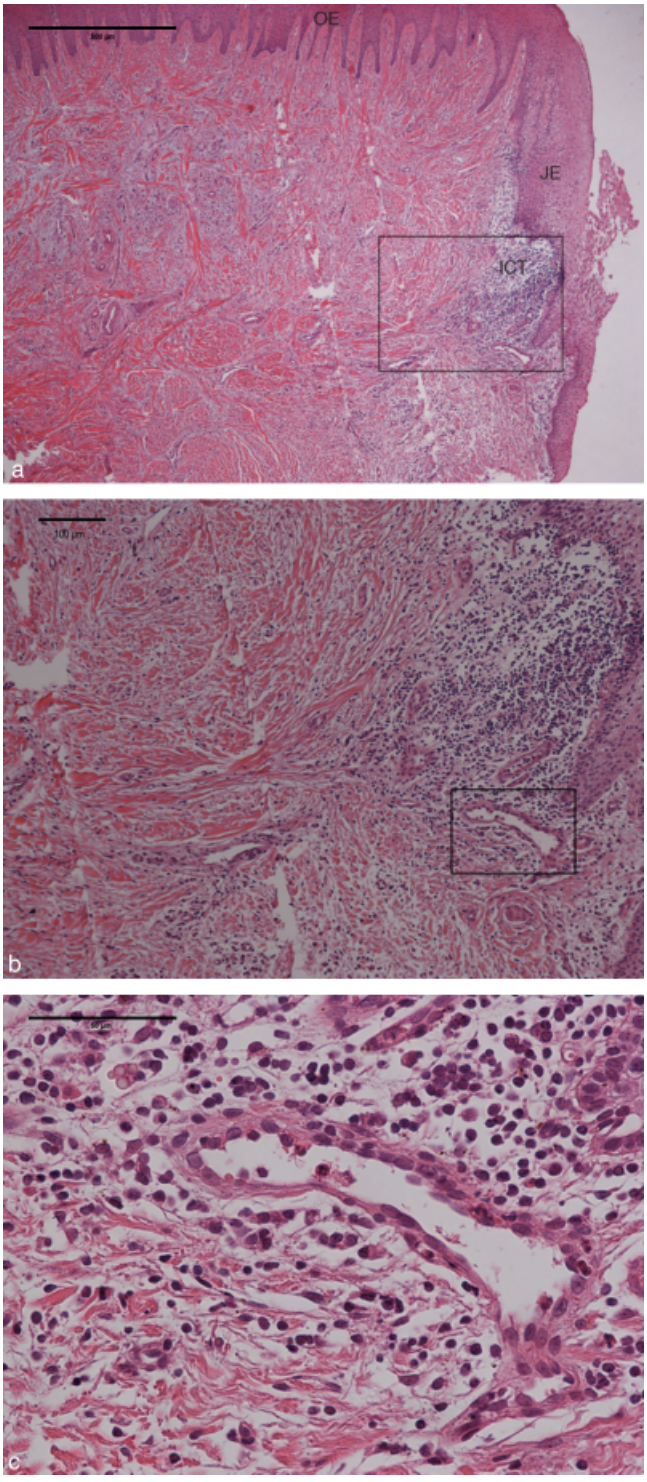

Fig. 3. (a) A sample of test group$_1$ (implant diameter 4.3 mm) stained with haematoxylin–eosin. A small and localized area of infiltrated connective tissue underlies the junctional epithelium. The remaining area of the connective tissue appears healthy and well organized. Original magnification × 40. (b) Higher magnification of Fig. 2a. The lymphocytes underlay the junctional epithelium. Original magnification × 100. (c) Higher magnification of Fig. 2b representing a large diameter vessel in the inflamed connective tissue below the junctional epithelium. T lymphocytes are the predominant cells in the infiltrated area. Original magnification × 200.

Below the JE, larger diameter microvessels surrounded by the ICT mononuclear cells were found (Fig. 3c).

The correlation coefficients between the MVD and the clinical variables (BOP and PD) resulted as not significant (Table 4).

AA% and organization of collagen fibres

As showed in Table 3, no differences in collagen content were evident between groups ($\chi^2 = 0.37$, $p = 0.95$).

At the evaluation with polarized light, the collagen fibres under the oral epithelium were thick and closely packed and appeared well oriented in a perpendicular structure of bundles (Fig. 5). In correspondence of JE where the ICT was localized, the collagen fibres were arranged in a thin, loose and disorganized structure, and also unstained areas appeared (Fig. 6).

The correlation coefficients between the collagen content and the clinical variables (BOP and PD) resulted as not significant (Table 4).

Discussion

In a previous parent study, Canullo et al. (2010) investigated radiographically the benefits of different mismatching diameter switching platform and observed that an increasing implant/abutment mismatching diameter resulted in an even better marginal bone preservation. It has been hypothesized that this clinical and radiographic advantage is consequent to the reduction of inflammation within the soft tissue and to the augmented biological width by moving the IAI horizontally away from the bone (Lazzara & Porter 2006), to the increased sealing property of the peri-implant connective tissue (Becker et al. 2007, Degidi et al. 2008) and to the better distribution of masticatory load.

In the present study, histological evaluations being conducted on the same sites that had been evaluated in the parent clinical study. Despite significant radiographic differences were found between control and test groups (Canullo et al. 2010), at the histological evaluation no significant differences appeared in terms of (i) amount and distribution of infiltrates of inflammatory cells, (ii) MVD and (iii) area fractions occupied by collagen fibres. These findings support the hypothesis of a positive effect of moving the IAI away from the bone more than the hypotheses

Table 3. Number of samples with well-preserved junctional epithelium (JE) is indicated; in these sites, the maximum area of inflammatory infiltrated cells (ICT) was calculated (mean and standard deviation)

Group	Samples with JE (*N*)	Mean (SD)		
		ICT area (mm^2)	MVD (%)	AA (%)
Control: 3.8 mm ($n = 11$)	2	0.26 (0.04)	10.7 (3.7)	63.2 (10.1)
1: 4.3 mm ($n = 10$)	5	0.13 (0.05)	8.6 (2.7)	61.8 (11.5)
2: 4.8 mm ($n = 9$)	4	0.12 (0.12)	11.3 (4.5)	63.6 (12)
3: 5.5 mm ($n = 7$)	6	0.18 (0.12)	12.4 (3.1)	64.6 (11.8)
p		NS	NS	NS

Microvascular density (MVD) and collagen content (AA%) were evaluated in all sites (mean and standard deviation).
Comparison: Kruskal–Wallis test; NS, not significant ($p > 0.05$).

Table 4. An overall correlation coefficient was calculated between clinical parameters [bleeding on probing (BOP), and probing depth (PD)] and area of inflammatory connective tissue (ICT), microvascular density (MVD) and collagen content (AA%)

	BOP	PD (mm)
ICT	0.37	0.07
MVD	− 0.09	− 0.27
AA%	0.05	− 0.17

Pearson's correlation coefficient: all values were not significant ($p > 0.05$).

of the reduction of inflammatory infiltration at the interface abutment/JE and the improvement of the resistance of the connective tissue.

At the sampling, all experimental sites included in the study were clinically healthy; however, at histological evaluation a small amount of inflammatory cells were localized in the connective tissue underlying the JE. No significant differences were found between the four experimental groups regarding the ICT size. Most of the cell populations were lymphocytes and only few polymorphonuclear cells were found. These findings are in agreement with previous studies. Inflammatory cells were detected in clinically healthy gingiva and peri-implant mucosa as well as in peri-implant mucositis and peri-implantitis (Tonetti et al. 1995, Gualini & Berglundh 2003). However, the volume of connective tissue occupied by these cells and the proportion of cell phenotypes are strictly correlated to the clinical symptoms of the inflammation and the long-term implant survival (Zitzmann et al. 2001, Gualini & Berglundh 2003). In clinical healthy gingiva and peri-implant tissues, lymphocytes, mostly T cells, were found in a narrow area of connective tissue lateral to the JE (Tonetti et al. 1995, Liljenberg et al. 1997). In the inflamed peri-implant mucosa and in peri-implantitis, the proportion of B cells gradually increases in spite of the number of T cells, and the area of connective tissue also gradually extends. In the present study, the phenotype characteristics of inflammatory infiltrated cells were not evaluated.

Evaluation of tissue vascularization is a further way to explore the tissue inflammation (Johnson et al. 1999). The MVD was used previously to assess the inflammation of the peri-implant soft tissue (Cornelini et al. 2001, Degidi et al. 2006) and was positively associated with tissue inflammation. Healthy mucosa showed a limited inflammatory infiltrate and vessels uniformly distributed in the connective tissue; at peri-implantitis sites, the vascularity was higher and the inflammatory infiltrate was more dense (Cornelini et al. 2001, Bullon et al. 2004). In the present study, blood vessels were identified by means of CD31-related antigen and the MVD was calculated with the stereological count of vessels. No signs of peri-implantitis (suppuration, deepened pocket and loss of supporting marginal bone) were found; only a physiologic crestal re-modelling was observed (Canullo et al. 2010).

High differences in vascular network were found within the same group, but mean values of ICT and MVD showed no significant differences between groups. Furthermore, no significant correlation was found between clinical parameters (BOP and PD) and histological data (ICT, MVD and AA%). Besides, the reduced values of BOP and PD in all sites may suggest an overall health condition, thus mismatching implant/abutment connection seems not to be influencing the long-term inflammation process of the peri-implant soft tissue. Hence, it is supported by the hypothesis that benefits from platform-switching technique on bone loss detected radiographically (Canullo et al. 2010) are consequent to a shift of the ICT away from the adjacent bone, rather than a reduced inflammatory effect of the platform-switched abutment within the surrounding soft tissue (Lazzara & Porter 2006).

In order to explore sealing properties of peri-implant connective tissue and biomechanical differences between implants using matching or mismatching implant–abutment connection, the area fraction and distribution of collagen fibres were evaluated. The mean area fraction occupied by collagen fibres was evaluated and ranged from 60% to 64% without significant differences between groups. Similar quantitative evaluation of collagen content was reported previously in healthy gingival tissue (Séguier et al. 2000, Ejeil et al. 2003). During peri-implant inflammation, the degradation of connective tissue matrix macromolecules and collagen fibres is induced by metalloproteinases, cellular infiltrate and resident cells (fibroblasts) (Séguier et al. 2000). Studies showed that from a status of health of the gingival/peri-implant connective tissue to a status of inflammatory disease, collagen content of the connective tissue progressively decreases (Séguier et al. 2000, Ejeil et al. 2003). Borsani et al. (2005) also observed that, in healthy peri-implant connective tissue, collagen fibres were well organized and generally homogeneous through the sample. However in inflamed peri-implant extracellular matrix, the collagen fibres were loosely packed, thin fibrils, disorganized and not well arranged, impairing the structural resistance of the soft tissue to bacterial penetration. From the evaluation of the distribution and the amount of collagen fibres, it appears that the localized ICT at the level of JE accords to the impaired status of collagen fibres in the same area, and that this situation is limited at the sub-JE area and does not extend within the outlying connective tissue.

Collagen bundle arrangement was observed with polarized light and appeared similar between groups. It was well organized in perpendicular bundles in the healthy peri-implant connective tissue, but became loose and disorganized around the ICT adjacent the JE.

The network of supracrestal collagen fibres might be of clinical relevance as a mechanical protection for the underlying bone–implant interface (Schierano et al.

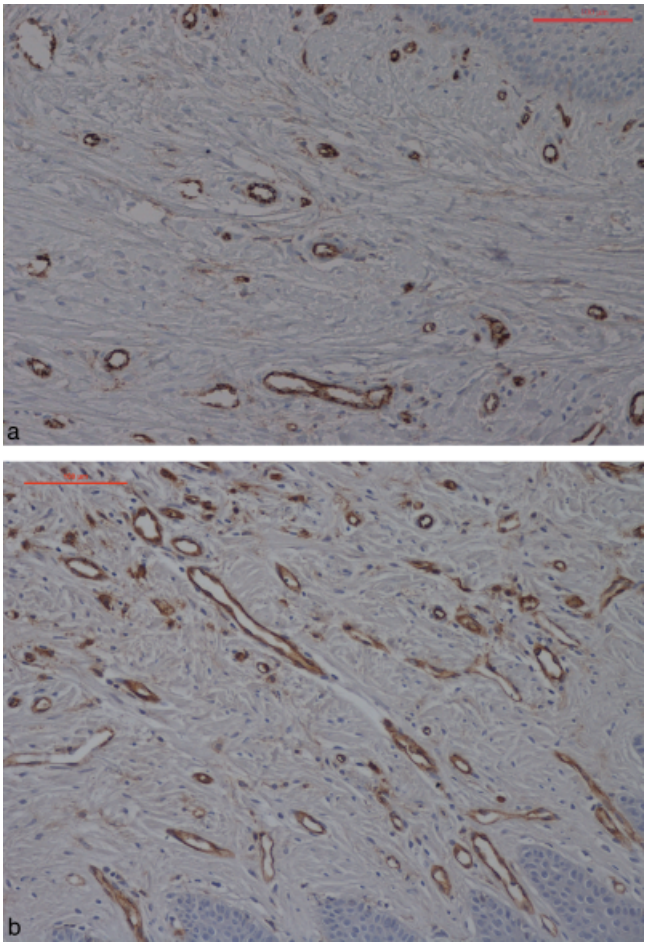

Fig. 4. Microphotograph of the connective tissue close to the oral epithelium. Sections of two different samples of test group$_3$ (implant diameter 5.5 mm) immunostained for CD31 antigen show the intra-group high variability of microvascular density [(a) low microvascular density, (b) high microvascular density]. Original magnification × 200.

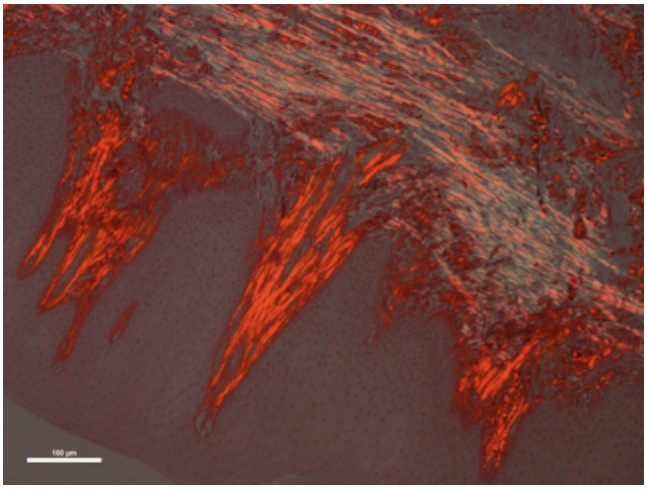

Fig. 5. Microphotograph of oral epithelium and underlying connective tissue of a sample of the test group$_3$ stained with Sirius red and observed with polarized light. Collagen fibres appear individually defined, well organized and perpendicularly oriented; a group of bundles run in the sub-epithelial area parallel to the surface of the mucosa, another group run from the connectival papillae to the inner area of the connective tissue, crossing the sub-epithelial bundles. Original magnification × 100.

2002). In a previous long-term human study (Schierano et al. 2002), it was observed that peri-implant collagen fibres orientation around implant-retained overdenture or implant-supported fixed restoration was constant, organized in three major systems (circular, longitudinal and oblique fibres) and may not depend on the type of prosthetic reconstruction and functional loading.

The ideal method to evaluate the biologic width and peri-implant soft tissue organization is the block section that presents obvious ethical concern, in particular if a large sample study want to be design. Even if the harvested samples were of small size and were not connected to the implant surface, the current findings support the evidence that different biomechanical characteristics of dental implant do not change the organization of collagen fibres in the soft tissue. Therefore, the hypothesis that switching platform lead to the establishment of a wider and more resistant area of connective tissue at the level of implant abutment connection seems to be refused (Becker et al. 2007, Degidi et al. 2008).

Unlike the soft tissue organization, collagen fibre orientation in peri-implant bone may change depending on loading distribution (Traini et al. 2005). In a 3D finite element model, switching platform configuration demonstrated a stress area shifted towards the centre of the implant compared with the traditional implant configuration, reducing the shearing stress at the bone–implant interface (Maeda et al. 2007). It was hypothesized that this better distribution of loading stress at the IAI reduces bone loss after implant restoration (Canullo et al. 2010).

Future studies on collagen fibre orientation in peri-implant bone tissue should be designed to better explain the biomechanical advantages of mismatching IAIs.

At a short time after implant restoration, inflammatory and damaging events occur to the peri-implant hard and soft tissues to reach the biological width (Hermann et al. 2000).

In the previous clinical study (Canullo et al. 2010), a higher bone loss was observed in traditionally restored implants than switching platform implants, and the more extensive marginal bone loss occurred within 9 months after restoration and after 2 years only minor changes in bone level could be observed.

However, in the present histological long-term study, no significant differ-

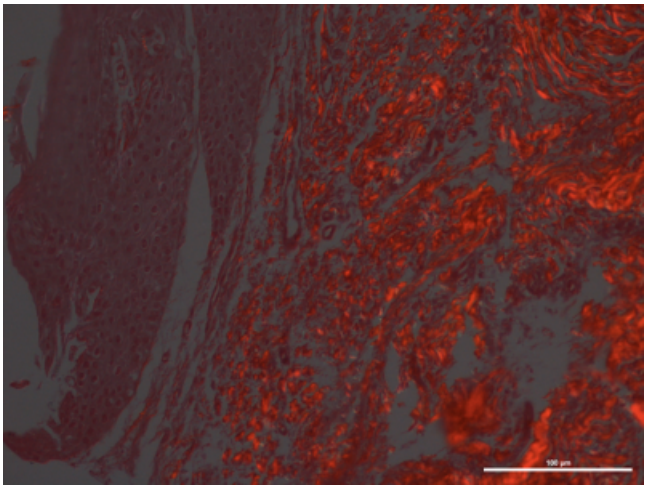

Fig. 6. Microphotograph of junctional epithelium and underlying connective tissue. Section of a sample of test group$_3$ stained with Sirius red and observed with polarized light. The collagen fibres in the area of infiltrated connective tissue, adjacent the junctional epithelium are loose, thin, dissociated and not well oriented. Unstained areas also appear. Original magnification $\times$ 200.

ences in terms of inflammatory rate of the soft tissue were found independently from the previously reported bone loss amount.

It may be speculated that shortly after implant restoration the damaging effects are stronger in matching than in switching restored implants, and after the re-establishment of the anatomical normal architecture, the soft tissue of all sites achieves the same trophism. Thus, to observe the inflammatory mechanism and verifying this hypothesis, a further similar short-term study should be designed.

Conclusions

The results of the present study indicate that 48 months after restoration the peri-implant soft tissue around test and control sites had similar histological characteristics. Thus, the hypothesis that around switching platform abutment the increased biological width exerted positive effects on bone preservation seems to be more relevant than those sustaining the increased sealing properties of the peri-implant connective tissue. Furthermore, the IAI design of switching platform seems not to represent a risk factor for long-term soft tissue inflammation.

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Clinical Relevance

Scientific rationale for the study: Although the literature emphasized appealing short-term radiographic outcomes of implants restored according to platform switching, a lack of evidence on histological soft tissue response in humans was noted.

Principal findings: Comparing the soft tissues around implants restored traditionally or according to platform switching after 4 years of prosthetic loading, the present human study showed same inflammatory response and histological composition in both groups.

Practical implications: The present study could suggest that implant restoration using the platform switching concept does not represent a risk factor for long-term soft tissue inflammation.