Collagen Fiber Orientation Around Machined Titanium and Zirconia Dental Implant Necks: An Animal Study

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Purpose: *To evaluate in vivo collagen fiber behavior around two different dental implant necks placed in the mandibular bone of adult pigs.* ***Materials and Methods:*** *Scanning electron microscopic (SEM) and profilometric analyses were performed on both types of implant necks to evaluate the different surface morphology. Ten dental implants with machined titanium necks and 20 implants with zirconia necks were inserted into the mandibles of five adult pigs. Three months later, the animals were sacrificed; samples from the peri-implant mucosa were obtained and prepared for histologic analysis. Evaluation of collagen fiber orientation in the connective tissue surrounding the implant necks was performed by polarized light microscopy. Inflammation in the peri-implant soft tissues was also measured via the Gingival Index.* ***Results:*** *Postoperative healing was uneventful; all implants, except for one of each type, were osseointegrated after 3 months. SEM and profilometric analyses confirmed that zirconia necks showed* R_a, R_q, *and* R_z *values that were lower than those seen around the titanium necks. Histologic observation indicated that collagen fiber orientation was similar for both types of implants. The majority of fibers showed a parallel or parallel-oblique orientation to the implant surface for all samples. Implants that were not osseointegrated, as determined by clinical evaluation, showed inflammatory infiltrate, whereas healthy connective tissue was found around all the other implant necks.* ***Conclusions:*** *Collagen fiber orientation was similar, regardless of implant material, demonstrating a predominantly parallel or parallel-oblique pattern. Moreover, zirconia, which is used as a transgingival collar on some implants, showed connective tissue adhesion that was similar to that seen on the machined titanium surface, but demonstrated limited plaque formation and may provide better esthetics.* Int J Oral Maxillofac Implants 2009;24:52–58

Key words: *collagen fiber orientation, dental implants, machined titanium neck, zirconia neck*

In vitro and clinical studies, evidence from which has been used extensively in the treatment of patients with artificial prostheses, have shown that titanium and its alloys are suitable for dental implants.[1–3] Titanium combines excellent mechanical properties and an effective biologic response when placed into bone tissue.[4] These features allow titanium to interlock with bone tissue in a way that allows the implant to properly withstand the masticatory forces transmitted to bone tissue.[5]

Recent studies have shown that good bone/implant contact may not be sufficient to obtain long-term success with dental implants; success is also dependent on the quality of the soft tissues around the dental implant neck and on the orientation of the peri-implant collagen fibers.[6,7] Around a natural tooth, the collagen fibers of the periodontal ligament are radially oriented to the dental surface in the cervical area, a direction that maximizes resistance to tensile forces.[8] In contrast, longitudinal and circumferential fibers, the axes of which are parallel or oblique to the implant surface, have been observed around the titanium neck in dental implants.[9] This different organization of the

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collagen fibers around dental implant necks means that the peri-implant mucosa is less effective in protecting the area from plaque-released factors.[10,11]

Several studies have been carried out to find new materials with physical and chemical characteristics that can improve the soft tissue integration with dental implants and provide a better response by the hard and soft tissues. Zirconia has mechanical and biologic properties that are similar to those of titanium, and it has excellent biocompatibility with the host tissues.[12,13] Zirconia-coated titanium biocompatibility was confirmed by recent in vitro studies; it enhances adhesion and proliferation of fibroblasts and osteoblastlike cells.[14] Furthermore, the results of in vitro tests of carcinogenicity and teratogenicity (cellular chromosome aberrations) were negative, and genotoxicity tests showed an absence of aberrations in chromosomic patterns in cells cultured on zirconium plates.[15] Zirconia surfaces have shown, both in vitro and in vivo, lower bacterial deposition than titanium.[16–18] This particular characteristic may ensure excellent results for the soft tissue/implant interface.

The aim of this study was to evaluate in vivo collagen fiber orientation around two different titanium dental implant necks: machined titanium and zirconia.

MATERIALS AND METHODS

Dental implants with a machined titanium neck (Oct-In implants, Ide@, TBR Group/Sudimplant, Toulouse, France) and dental implants with a zirconia neck (Z1 implants, Ide@, TBR Group/Sudimplant) were used. Scanning electron microscopic (SEM) analysis was performed on one implant of each type to evaluate the neck surface morphology (Leo 435 VP microscope, LEO Electron Microscopy, Cambridge, UK) at 15 kV in high vacuum mode. The images were stored in TIFF format with a 1,024 × 768 pixel grid. Roughness measurements were performed on both types of implants and evaluated with the Leo 435 VP SEM and a Mitutoyo Surftest 211 Profilometer (Mitutoyo Corporation, Tokyo, Japan). An average of three readings was performed for each surface for determining the roughness parameters R_a (arithmetic mean deviation of the roughness profile), R_q (root-mean-square roughness), and R_z (peak-valley mean distance). A total of four implants (two zirconium and two titanium) were analyzed. Two areas of 200 μm in diameter, at the level of the implant neck, were observed for each implant type. An analysis of variance was applied as well as the Tukey post hoc comparative test to the R_a parameter to statistically compare the differences in roughness between sample groups.

Ten implants with the machined titanium neck and 20 with the zirconia neck were inserted in the mandibular crestal bone of five adult swine. All animal procedures were approved by the Ethics Committee of "G. D'Annunzio" University, Chieti-Pescara, and legal authorities. Mandibular premolars and molars were extracted under general anesthesia. After a 3-month alveolar bone-healing period, three implants, one with the machined titanium neck and two with the zirconia neck, were inserted on each side of the mandible in every animal under general anesthesia using a one-stage flapless surgical procedure, and the implants were not functionally loaded. Oral hygiene was performed on the day of surgery before implant insertion and monthly during the entire experimental period (3 months). Moreover, probing depth around each implant was measured once a month using a round periodontal probe (Michigan Probe, Ann Arbor, MI). The mean probing depths were calculated by averaging the readings from all the implants in each group. The presence of inflammation in the peri-implant mucosa was determined by using the Gingival Index (GI), as described by Loe.[19] The clinical data were always collected by the same operator.

The animals were sacrificed 3 months after insertion of the implants. Each mandible containing the implants was block sectioned, examined radiographically to evaluate bone healing around the implants, and then immersed in a fixative solution of formalin 4%. The specimens were then dehydrated with gradually increasing concentrations of ethanol and xylene. Samples were then infiltrated with several changes of polymethylmethacrylate (PMMA) monomer, followed by polymerization of the PMMA, as previously described.[20] Specimens of the peri-implant mucosa were collected by a surgical blade. Thin sections were saw-sectioned and ground to approximately 100 μm for light microscopic analysis to evaluate collagen fiber orientation on the implant necks tested. Histologic analysis was performed in the connective tissue area and at the epithelium–connective tissue junction. Sections were stained with hematoxylin-eosin and examined under an Axiolab microscope (Zeiss, Oberkochen, Germany) connected to a digital camera (Fuji FinePix2; Fujifilm Corporation, Tokyo, Japan). The images were stored in TIFF format with a grid of 1,024 × 768 pixels.

To determine the collagen fiber orientation around the implant necks, the angles of the fibers attached to the surface were measured using polarized light microscopy. The mean distributions (percentages) of collagen fiber orientation were calculated by dividing the attachment length of a particular angle group by the total length of the

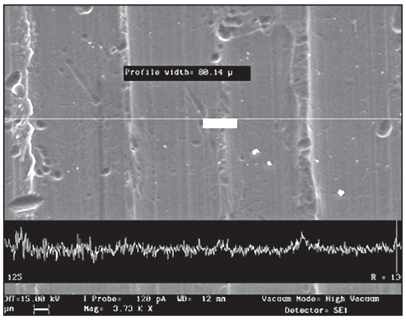

Fig 1 SEM and profilometric evaluation of machined titanium neck surface of an Oct-In dental implant (magnification ×3,730).

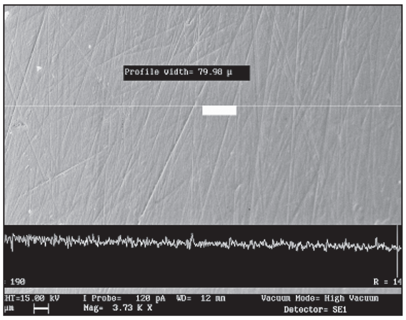

Fig 2 SEM and profilometric evaluation of zirconia neck surface of a Z1 dental implant (magnification ×3,730).

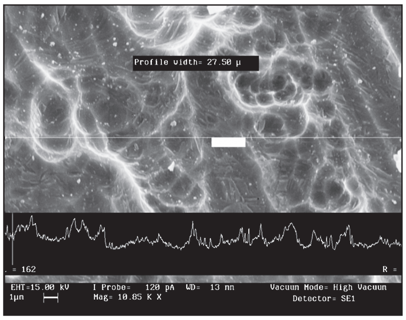

Fig 3 SEM and profilometric evaluation of zirconia surface of a Z1 dental implant. The superficial mean roughness of zirconia was always less than 1 µm (magnification ×10,850).

Table 1 Clinical and Histologic Summary of the Implants

Type of implant	No.	GI score	Soft tissue clinical findings	Mean probing depth	Histologic findings
Osseointegrated implants					
Z1	19	0–1	Slight recession No visible inflammation	2.0 ± 0.2 mm	Absence of remarkable inflammatory cells
Oct-In	9	0–1	Slight recession No visible inflammation	2.2 ± 0.2 mm	Absence of remarkable inflammatory cells
Nonintegrated implants					
Z1	1	2	Erythematous gingiva Bleeding on probing	4.7 ± 0.2 mm	Remarkable presence of polymorphonuclear neutrophils and lymphocytes
Oct-In	1	2	Erythematous gingiva Bleeding on probing	4.6 ± 0.2 mm	Remarkable presence of polymorphonuclear neutrophils and lymphocytes

zone, and these were classified using the following criteria, modified from Comut et al[21]: 0 to 10 degrees = parallel; 11 to 30 degrees = parallel-oblique; 31 to 60 degrees = oblique; 61 to 85 degrees = oblique-perpendicular; and 86 to 90 degrees = perpendicular. The differences among data of collagen fibers with various orientations between the titanium implant surface and the zirconium surface were evaluated with the data analysis software Statistica 8 (StatSoft Inc, Tulsa, OK). Data were expressed as a percentage after the normalization of values. The *t* test was performed on the results, and *P* values above .05 were considered statistically insignificant.

RESULTS

The machined titanium and zirconia necks were observed by SEM, and the surface morphology was analyzed by profilometry (Figs 1 to 3). The different implant types demonstrated a few differences in surface roughness; R_a, R_q, and R_z values, which describe the average deviation from the mean line, were 0.87 ± 0.09 µm, 20.4 ± 1.30 µm, and 2.36 ± 0.65 µm, respectively, for titanium versus 0.56 ± 0.11 µm, 18.64 ± 1.05 µm, and 1.89 ± 1.43 µm, respectively, for the zirconia surface.

Postoperative healing was uneventful for all animals. Clinical and radiographic evaluations showed that 28 of the inserted implants were anchored in the bone. Only one implant with a machined titanium neck and another with a zirconia neck had not osseointegrated. The mean probing depth recorded around osseointegrated implants throughout the experimental period was 2.2 ± 0.2 mm for implants with the polished titanium neck and 2.0 ± 0.2 mm for dental implants with the zirconia neck. Soft tissues around most of the implants showed slight recession with no visibly present inflammation (GI = 0 or 1 in all osseointegrated implants). In contrast, the peri-implant mucosa around nonintegrated dental implants appeared erythematous and exhibited bleeding on probing. Among these, a mean probing depth of 4.6 ± 0.2 mm and a GI score of 2 were recorded around the implant with the machined titanium neck, while the nonintegrated zirconia-neck implant showed a mean probing depth of 4.7 ± 0.2 mm and a GI score of 2 (Table 1).

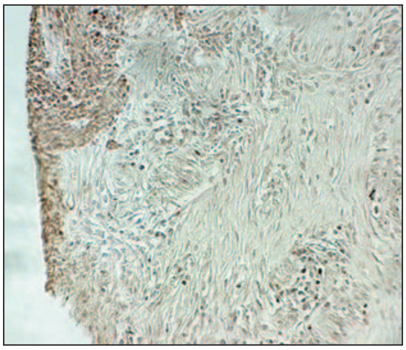

Fig 4 Histologic evaluation of a specimen from the connective tissue surrounding the nonintegrated Z1 implant. A moderate inflammatory infiltrate, with some neutrophils and lymphocytes, could be detected (hematoxylin-eosin; magnification ×4).

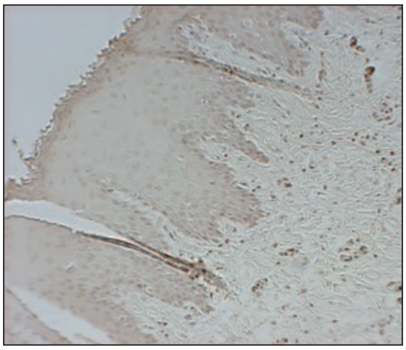

Fig 5 Histologic evaluation of a specimen from the connective tissue surrounding an osseointegrated Z1 implant. In some areas collagen fibers were disorganized and had no discernible orientation in relation to the implant neck (hematoxylin-eosin, magnification ×4).

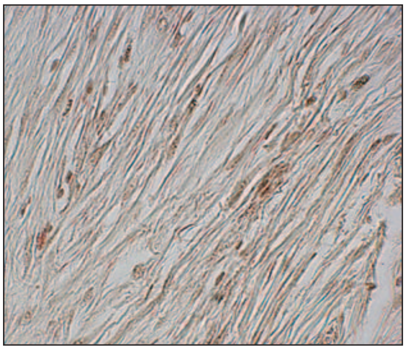

Fig 6 Histologic evaluation of a specimen from the connective tissue surrounding an osseointegrated Z1 implant. In some areas collagen fibers were disorganized and had no discernible orientation in relation to the implant neck (hematoxylin-eosin, magnification ×20).

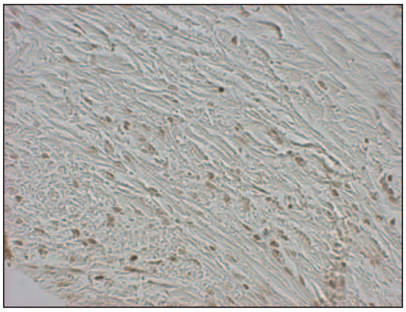

Fig 7 Histologic evaluation of a specimen from the connective tissue surrounding an osseointegrated Z1 implant. Areas with few collagen fibers showed a higher number of fibroblasts (hematoxylin-eosin, magnification ×10).

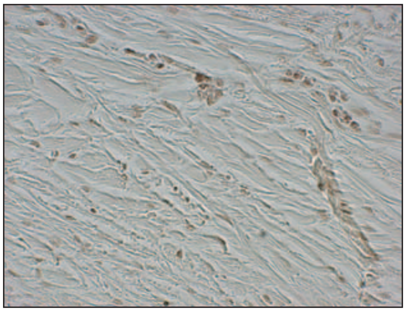

Fig 8 Histologic evaluation of a specimen from the connective tissue surrounding an osseointegrated Z1 implant. Fewer fibroblasts were seen in the areas of organized collagen bundles (hematoxylin-eosin, magnification ×10).

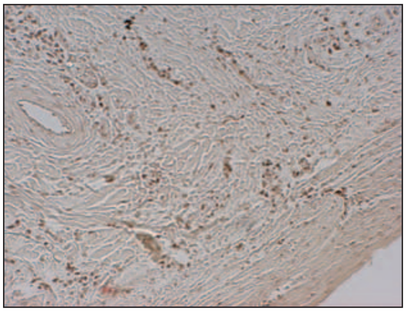

Fig 9 Most of the fibers were longitudinally oriented to the zirconia dental implant neck, as was the case for the smooth titanium dental implant neck (hematoxylin-eosin, magnification ×4).

At histologic evaluation, the peri-implant mucosa collected around osseointegrated implants showed an insignificant inflammatory infiltrate. Many inflammatory cells (polymorphonuclear neutrophils and lymphocytes) could be detected in the specimens from nonintegrated implants (Fig 4). In some areas, the peri-implant mucosa connective tissue was well organized, with fibers approaching the implant surface in a definite direction, while in some other areas it was disorganized, with an unclear fiber orientation (Figs 5 and 6). Areas of organized collagen bundles showed few fibroblasts, whereas many fibroblasts were seen in the regions with few fibers (Figs 7 and 8). In some cases, the fibers showed the same orientation and direction throughout the connective tissue, whereas in other cases they changed their orientation and direction along the surface; in some cases, fibers with opposite directions overlapped each other (Figs 9 and 10).

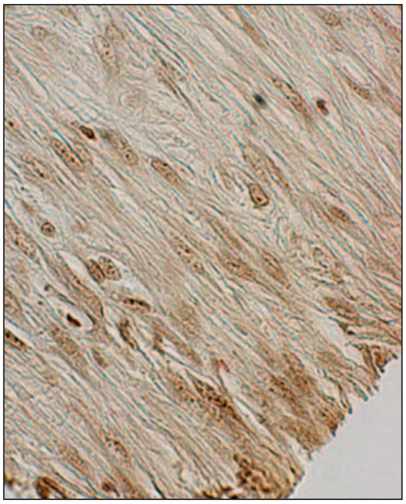

Fig 10 Histologic evaluation of a specimen from the connective tissue surrounding an osseointegrated Z1 implant. A low percentage of collagen fibers approached the implant surface in an oblique or perpendicular orientation (hematoxylin-eosin, magnification ×20).

Table 2 Distribution (%) of Collagen Fiber Orientation for All Implants

Surface	0–10 deg (parallel)	11–30 deg (parallel-oblique)	31–60 deg (oblique)	61–85 deg (oblique-perpendicular)	86–90 deg (perpendicular)	Unclear orientation
Z1	16%	18%	14%	6%	5%	41%
Oct-In	19%	22%	17%	5%	4%	33%
Z1 (nonintegrated)	82%	5%	0%	0%	0%	13%
Oct-In (nonintegrated)	80%	7%	0%	0%	0%	13%

Table 3 Results of *t* Test

Surface	0–10 deg + 11–30 deg Z1	0–10 deg + 11–30 Oct-In	61–85 deg + 86–90 deg Z1	61–85 deg + 86–90 deg Oct-In
0–10 deg + 11–30 deg Z1	-	NS ($P > .05$)	$P = .0412$	ND
0–10 deg + 11–30 deg Oct-In	NS ($P > .05$)	-	ND	$P = .0395$
61–85 deg + 86–90 deg Z1	$P = .0412$	ND	—	NS ($P > .05$)
61–85 deg + 86–90 deg Oct-In	ND	$P = .0395$	NS (P > .05)	—

ND = not determined: These different groups of fibers on different implant necks were not evaluated; NS = not significant.

The evaluation of collagen fiber orientation was performed in different areas of the connective tissue surrounding the implant necks. The attachment percentages were calculated by dividing the attachment length of a particular angle group by the total length of the zone and following the criteria described. Around the zirconia neck implants, most of the collagen fibers (48%) showed a parallel, parallel-oblique, or oblique orientation; 16% of them were parallel, 18% were parallel-oblique, and 14% were oblique. Only 11% of them were oblique-perpendicular (6%) or perpendicular (5%) oriented, whereas a high percentage (41%) included unoriented fibers. The percentage distribution for the machined titanium neck implants was quite similar: a high percentage of parallel, parallel-oblique, and oblique fibers (58%) was found, despite lower percentages of oblique-perpendicular and perpendicular (9%) and unoriented collagen fibers (33%). In the two nonintegrated implants, the dominant fiber orientation observed was 0 to 10 degrees. Neither implant showed collagen fibers oriented at 31 to 90 degrees (Table 2).

No statistically significant differences in parallel or parallel-oblique fiber orientation were found between the different neck types. Analysis of oblique-perpendicular and perpendicular collagen fibers showed the same results ($P > .05$). A significant difference among fibers was found only between parallel and parallel-oblique groups with respect to perpendicular and oblique-perpendicular groups of the same specimen type (Table 3). All the specimens showed clinically healthy connective tissue around all implants.

DISCUSSION

The main aim of this investigation was to evaluate the behavior of the collagen fibers in the connective tissue around the dental implant necks of two different implant types: one with a machined titanium neck and another with a zirconia neck. Long-term implant survival rates and maintenance of implant osseointegration have been demonstrated to be influenced by peri-implant soft tissue health.[8,9] For this reason, the surface characteristics of the implant neck play an important role in a strategic area of deep tissue remodeling, by creating a biologic width and influencing plaque control.[6]

A correlation between plaque accumulation and progressive bone loss around implants has been reported in experimental and clinical studies, and this is considered a critical factor for success in implant rehabilitations.[22–24] For this reason, a coating for a dental implant neck that helps reduce plaque accumulation and guarantees a good seal against plaque-releasing factors would be desirable. During preliminary SEM and profilometric analyses, the zirconia surface showed roughness values that were lower than those on the titanium surface. According to the opinion that less plaque adheres to smoother surfaces,[18,25] the zirconia surface would seem to promote less plaque adhesion. The in vivo clinical evaluation confirmed this by finding no signs of inflammation or plaque accumulation in osseointegrated implants, with a mean probing depth that was lower on the zirconia neck implants than on dental implants with the machined titanium neck.

The extracellular matrix is increasingly being identified as playing a complex and important role in many biologic processes, including the peri-implant soft tissue/implant relationship. Collagen fibers are the major component of the extracellular matrix in all mammalian connective tissues; they form an essential framework used by fibroblasts as scaffolding to "crawl" along.[26] Thus, collagen fiber orientation influences the direction of fibroblast growth and the fibroblasts' ability to move toward the implant neck and form an adequate connective seal. At clinical evaluation and in the histologic results, no signs of inflammation were found around the osseointegrated implants, confirming that zirconia is as compatible with the host tissues as titanium. Similar connective tissue adhesion was observed in implants with both types of neck. Most of the fibers were parallel-oblique and parallel to the implant surface, unlike natural teeth, where collagen fibers insert into the bone and cementum occurs as they are entrapped as mineralization ensues.[27] The percentage of parallel fibers (0 to 10 degrees and 11 to 30 degrees in Table 2) was statistically significantly different from the percentage of perpendicular fibers (61 to 85 degrees and 86 to 90 degrees in Table 2) for each implant type. The statistical analysis showed that in both implant necks, a percentage of perpendicular fibers was present, but this was not a determining factor between zirconia and Oct-In specimens. A lower percentage of oblique-perpendicular and perpendicular collagen fibers approaching the zirconia neck was found compared with Oct-In, but this difference was not statistically significant (Table 3).

CONCLUSION

This study showed that a zirconia neck seems to ensure tissue healing that is clinically and histologically comparable to that seen around a machined titanium neck. Around implants with the zirconia neck, the recorded mean probing depth, less than around implants with the machined titanium neck, is compatible with a minor inflammatory response. This important point, combined with the hypothesized lower plaque formation because of the smoother surface of zirconia, is vital for obtaining a good soft tissue/implant correlation and a good esthetic result. Statistical analysis of the difference between fiber orientation on the two types of implants also showed that there were no substantial advantages in the use of traditional machined necks; most of the collagen fibers around implants were oriented parallel and oblique-parallel to both types of implant necks. The results also suggest that further in vivo investigations must be performed to best characterize the host tissues' responses to zirconia and also to evaluate the peri-implant connective tissues' behavior under different implant loading conditions.

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