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Journal of Applied Biomaterials & Biomechanics 2004; 2: 143-150

In vitro and *in vivo* follow-up of titanium transmucosal implants with a zirconia collar

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ABSTRACT: *The advantages of transmucosal healing implants with a bioactive zirconia collar as a support for partially fixed prosthodontic restorations are optimal peri-implant marginal tissue sealing, reduction in plaque accumulation and satisfactory esthetic results. The zirconia used in this study evidenced not only optimal clinical performances, but also good biocompatibility. The results from this study demonstrated that zirconia coating enhances fibroblasts and osteoblast-like cell adhesion, spreading and proliferation, favoring microscopic tissue/cell in-growth and clinical implant fixation improvement. From clinical analysis, it emerged that the treatment group obtained better scores in every peri-implant parameter. This evidence attests faster stabilization of soft and hard tissues around both the transmucosal zirconia collar and at the crestal level of the implant. A reduced plaque accumulation around the implant with zirconia collar could provide a better peri-implant microbiological environment by allowing the soft tissues expression of optimal sealing and good bone adaptation to loading. From these clinical and radiographic comparative analyzes, it emerged that in the treatment group the mean values were always similarly low. A rapid stabilization of both hard and soft peri-implant tissues was documented in the 1st yr. In the treatment group, there was the formation of stable tissue sealing the zirconia collar, which could preserve mucosal and bone levels. In conclusion, 2-yr clinical results demonstrated that implants supporting fixed restorations using transmucosal healing implants with a zirconia collar appeared a valid method, reporting 100% implant survival rates. Moreover,* in vivo *results obtained using strict parameters to assess the peri-implant status affirmed that a zirconia collar offers excellent biological acceptance. Our preliminary* in vitro *results statistically evidenced increased fibroblast and osteoblast adhesion and proliferation to zirconia compared to titanium, and an index of enhanced material integration with bone and soft tissue cells. (Journal of Applied Biomaterials & Biomechanics 2004; 2: 143-50)*

KEY WORDS: *Osteoblast like cells, Osteointegration, Zirconia*

Received 04/01/04; Revised 25/01/04; Accepted 04/11/04

INTRODUCTION

The improved understanding in material-cell interactions results in a high degree of predictability in the clinical success of biomaterials used for dental implants (1, 2). Titanium has been established as the first choice material for endosseous implants because of its chemical-physical properties and its biocompatibility (3, 4).
Restorations in the anterior esthetic zone present significant challenges in both the surgical and prosthetic phases of implant dentistry and many types of implants require transmucosal abutments to retain implant restorations. In these cases, the gray color of the titanium is transmitted through the peri-implant tissues causing patient discomfort.
The use of ceramic abutments in methods with submerged implants allows the minimization of the gray color associated with metal components. Submerged implants, on the other hand, evidenced limits connected to the operative phases: a second surgical step, longer clinical times, and the irreversibility of the prosthetic structure. Therefore, some previous studies compared non-submerged healing implants, also defined as one-stage implants or transmucosal healing implants, with submerged healing implants, also defined as two-stage implants. These studies, found no differences in

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the long-term prognosis of endosseous dental implants (5, 6), promising important clinical advantages for the one-stage surgery technique.
The use of a new implant with a white transmucosal ceramic collar allowed the combination of the advantages offered by transmucosal implant methods with a one-stage technique with natural transparency and increased biocompatibility at the transmucosal portion.
Zirconia is an advanced ceramic used in the bone medical field (7) due to its important mechanical properties and tissue biocompatibility. In addition, zirconia has been shown to reduce bacterial adhesion (8) and plaque accumulation (9).
In this study, we evaluated *in vitro* the biocompatibility of titanium compared to zirconia coated titanium by studying fibroblasts and human osteoblast-like cell adhesion and proliferation. In addition, we performed preliminary clinical evaluations of transmucosal healing implants with and without a zirconia collar.

MATERIALS AND METHODS

Materials

Two types of solid screw transmucosal healing implants with rough endosseous surfaces (Z1 implants, T.B.R.® ide@, Sudimplant, Toulouse, France) were used. For both types, the endosseous surface was titanium, while the transmucosal collar was titanium or titanium covered by a zirconia ring. The ceramic ring was a yttria stabilized medical grade zirconium dioxide.
The implants used for the clinical studies, in relation to bone height disposal, had an intraosseous length ranging from 10.5 to 15.5 mm, and concerning the bone width disposal, had an intraosseous diameter from 3.5 to 5 mm. For *in vitro* tests, titanium and zirconia disks of 0.4 cm diameter were used. Prior to use, the coated and uncoated materials were sterilized for 2 hr in dry heat at 160 °C.

In vitro e*valuations*

Cell behavior on the materials tested was studied using human fibroblasts (MRC5, ATCC cell-line) and human primary osteoblast-like cells obtained by enzymatic isolation from trabecular fragments of adult human bone removed during surgery, and treated as described previously (10). Briefly, particles of 3-5 mm, after treatment with bacterial collagenase, were plated in 90 mm tissue culture dishes and cultured at 37 °C in 95% air / 5%CO_2 in 10 ml ISCOVE's supplemented with 20% FBS, 50 U / ml penicillin, 15 µg / ml streptomycin and 2 mM glutamine. Cell outgrowths from the bone fragments appeared within 1 week and formed a confluent monolayer at 3-4 weeks. The isolated bone cells were characterized including osteoblastic morphology, alkaline phosphatase expression and hormone responsiveness (parathyroid hormone, 1,25$(OH)_2D_3$). Fibroblasts and osteoblasts were used at a cell density of 1 x 10^4 cells / cm^2.
Cell adhesion and proliferation results were compared to that obtained on a polymeric substrate used as a control (Thermanox® slides, Nunc, Milano, Italy) known to induce cell adhesion (11).
As the materials tested were unsuitable for normal transmitted light microscopy, we observed cell morphology and distribution with fluorescence microscopy. After 6 hr (cell adhesion study) and 4 days (cell proliferation study), fibroblasts and osteoblast-like cells on the materials tested were rinsed in phosphate buffered saline, fixed for 20 min at 60 °C and stained for 5 min in a 0.025% acridine orange solution, a nucleic acid staining (12). Cell morphology and the number on each material were evaluated using a fluorescent microscope Aristoplan (Leitz Leica, Milano, Italy). Cell number was evaluated on a surface of 0.1715 mm^2 using 25x microscope magnification and results are reported as mean ± standard deviation of 30 fields obtained from three different experiments.

Clinical evaluation

A 2-yr randomized study was performed from 2000-2002. Twenty patients were admitted to the trial, and 44 implants were placed; 29 implants had zirconia collars (Z1 implants, T.B.R. ide@, Sudimplant, Toulouse, France) and 15 implants had standard titanium collars. Thirteen patients received only the implants with a zirconia collar, while five patients received implants with the same shape but with a titanium collar. The remaining two patients were implanted with both implants. Data from patients who received implants with a zirconia collar were processed as the treatment group, while data collected from patients who received implants with titanium collar were analyzed as the control group. The number of implants placed in the maxilla was 24, and 20 implants were placed in the mandible. The implants were used to support partially fixed prosthesis and underwent loading at 3-4 months after their placement.
The cumulative survival rate was calculated according to the method described by Cutler and Ederer (13). The establishment of successful outcomes re-

quired the periodical evaluation of specific parameters, such as the stability of peri-implant crestal bone levels and the health and stability of peri-implant soft tissue. According to Brägger et al (14), crestal bone levels were evaluated calculating the linear distance implant-shoulder to the bone (DIB), measured with periapical radiographs on mesial and distal sites (Fig. 1). When the DIB value is <3.5 mm, peri-implant bone is considered stable; >3.5 mm bone crest resorption has occurred.
The soft tissue status was evaluated clinically by the method proposed by Mombelli et al (15) for transmucosal healing implants and the following measures were recorded (Fig. 1).
Plaque index (PLI): This index is used to measure the level of patient oral hygiene performance; it confirms the quantity of bacterial deposits around the implant emerging from soft tissues. Value 0 = no plaque deposit, value 1 = small plaque deposit, value 2 = large plaque deposits.
Bleeding on probing (BOP): This index is used to measure the inflammation level of the mucosal tissues in response to peri-implant sulcus probing. Value 0 = no bleeding occurred during the probing, value 1 = small mucosal bleeding occurred, value 2 = significant bleeding occurred and value 3 = spontaneous bleeding, even if the mucosal sulcus has not been probed.
Probing depth (PD): This index measures (in mm) the mucosal sulcus depth around the implant. A small thin calibrated linear probe is inserted in the peri-implant sulcus with a pressure of only 25 g. As a starting point for the measurement, the free margin of the peri-implant mucosa is used, and an endpoint is reached when the resistance of the soft tissue does not allow further probing. When the PD value is <3 mm, peri-implant tissues are considered healthy; >3 mm mucosal pathology has occurred.
Probing attachment level (PAL) is related to the implant shoulder. An analogous procedure of PD measurement, but as a starting point the platform of the implants is used. When the PAL value is <2.5 mm, peri-implant tissues are considered healthy; >2.5 mm mucosal pathology and bone resorption have occurred.
Regular follow-ups from 4-6 months were established based on individual oral hygiene performance, but clinical data considered in this study were only those recorded annually.

Statistical analysis

Statistical analysis of the data was carried out using SPSS for Windows software. The Student's t-test for independent data was performed to compare adhesion and proliferation results. A p value was obtained from the ANOVA table; the conventional 0.05 level was considered statistically significant.

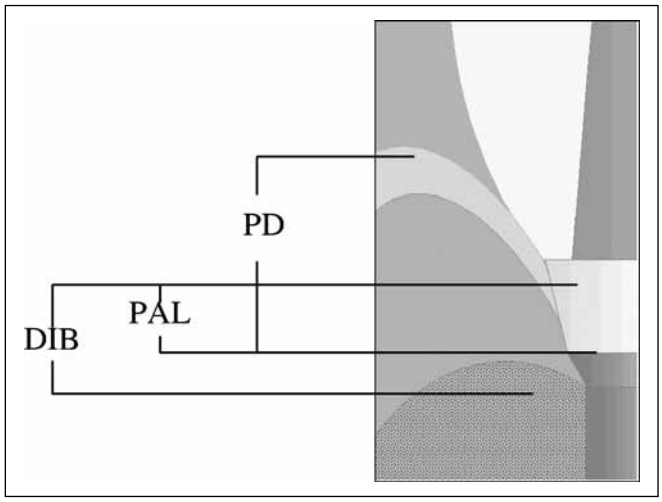

Fig. 1 - *Illustration depicting reference point and distances of clinical and radiographic measurements: PD (probing depth), PAL (probing attachment level, related to implant shoulder), DIB (distance implant shoulder/first implant bone contact).*

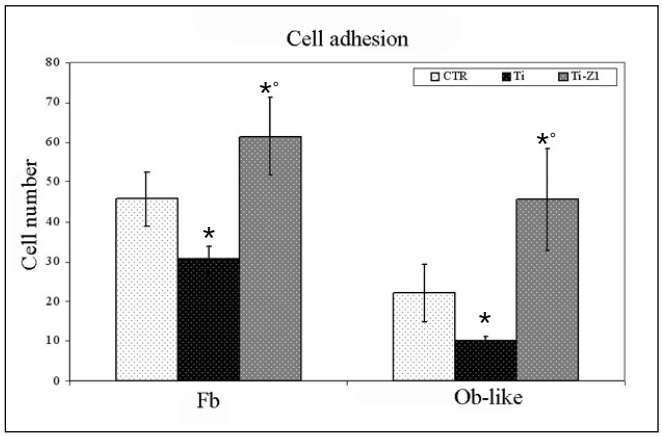

Fig. 2 - *Fibroblasts and osteoblast-like cells quantification at 6 hr cell incubation (adhesion test) on titanium (Ti), zirconia coated titanium (Ti-Z1) and on control Thermanox® slide (CTR). Cell number was the mean of 20 measurements in three experiments (n=60) and was referred to a surface area of 0.1715 mm². * p<0.05 with respect to controls; ° p<0.05 with respect to Ti.*

RESULTS

In vitro *results*

As shown in Figure 2, fibroblasts and osteoblast-like cells evidenced statistically higher cell adhesion when cultured on zirconia compared to the controls (Thermanox® slides) and compared to the uncoated titanium material.

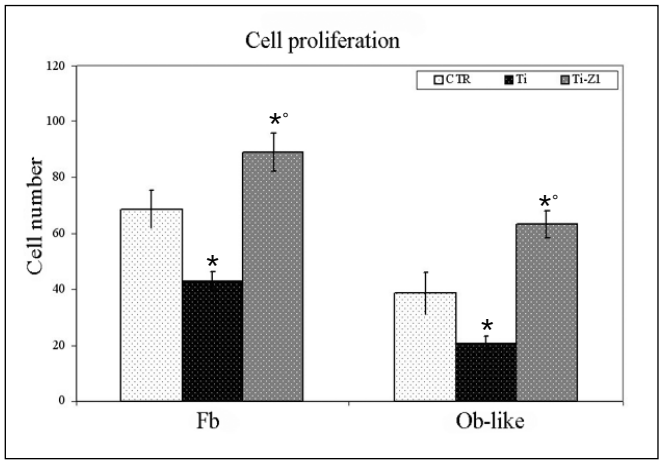

Fig. 3 - ***Fibroblasts and osteoblast-like cells quantification at 4 days cell incubation (adhesion test) on titanium (Ti), zirconia coated titanium (Ti-Z1) and on control Thermanox® slide (CTR). Cell number was the mean of 20 measurements in three experiments (n=60) and was referred to a surface area of 0.1715 mm². * p<0.05 with respect to controls; ° p<0.05 with respect to Ti.***

Figure 3 shows the results of fibroblasts and osteoblast-like cells at 4 days proliferation on the materials tested. Results evidenced increased cell numbers on the titanium and zirconia coated titanium with respect to the adhesion experiments, comparable to that obtained at 6 hr.

Human fibroblasts (Fig. 4a) and osteoblast-like cells (Fig. 4b) cultured on the surfaces of the two materials and observed at 6 hr incubation showed good fibroblast and osteoblast-like cell spreading on zirconia coated titanium, comparable to the control Thermanox® wells.

Clinical results

During the 2-yr observation, no implant was lost either in the treatment group or in the control group. In the follow-up period, all implants showed no mobility denoting clinical signs of stable osseointegration; therefore, an implant survival rate

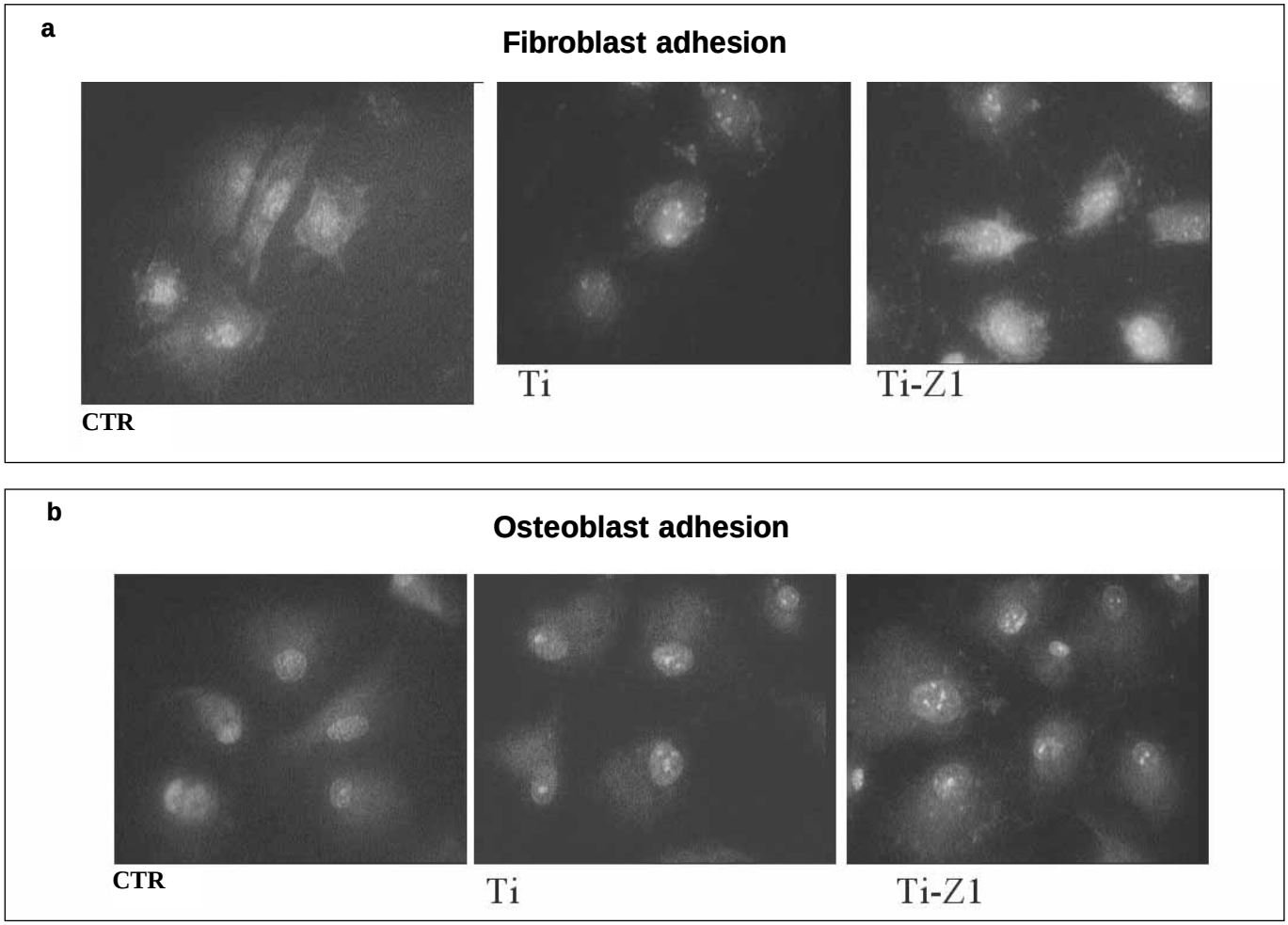

Fig. 4 - ***Morphology of fibroblasts (a) and osteoblast-like cells (b) adhesion on cell culture dish, titanium and zirconia coated titanium. Materials were incubated for 6 hr with fibroblasts and human osteoblast-like cells. Samples were examined by fluorescence microscopy at 250× magnification.***

of 100% was confirmed. Table I depicts the longevity of the implants, showing interval and cumulative survival rates both for the treatment and control groups.
Many patients exhibited good oral hygiene performance, and in most patients, crowns or partially fixed dentures were free from plaque or calculus deposits. Recorded peri-implant parameters demonstrated a PLI of 0 at 64.5% of all sites, and 1 at 29.5% and 2 at 6% of the other sites, respectively. During the observation period, analogous mean results were recorded in both groups, with a small percentage increase in the scores for the treatment group (Tab. II). Table II reports the differences in the mean values of BOP at the final evaluation. The BOP scores in the treatment group appeared lower as compared to the control group.
In both procedures, a slight increase in PD mean values, from 2.5 mm to approximately 3.5 mm, was encountered during the 1st yr of load application (Tab. III). Taking into consideration that the healthy conditions of the soft tissue does not admit a PD up to 3 mm, an analysis of PD for mean scores >3 mm was required. The data revealed better mean values of PD scores in the treatment group during the 2-yr interval suggesting a faster stabiliza-

TABLE I - LIFE TABLE ANALYSIS FOR 44 IMPLANTS SURVIVAL

Interval months	N° of implants at start of interval			Drop-outs during interval			Implants under risk			Failure during interval			Survival rate within period (%)			Cumulative survival rate (%)		
	Total	Treat*	Cont**	Total	Treat	Cont	Total	Treat	Cont	Total	Treat	Cont	Total	Treat	Cont	Total	Treat	Cont
12	44	29	15	2	2	0	42	27	15	0	0	0	100	100	100	100	100	100
24	9	5	4	0	0	0	9	5	4	0	0	0	100	100	100	100	100	100

* Treatment group ** Control group

TABLE II - PLI AND BOP INDEXES, PERCENTAGE OF MEAN VALUES AT LAST CONTROL

	Collar	Value 0	Value 1	Value 2
PLI	Zirconia	72	24	4
	Titanium	50	40	10
BOP	Zirconia	88.9	8.3	2.8
	Titanium	53.3	36.7	10

Mean values of PLI and BOP were significantly lower in treatment group

TABLE III - PERI-IMPLANT MEASURES RELATED TO OBSERVATION PERIOD, MEAN VALUES IN MILLIMETERS

	Groups	Time period 0 months	Time period 6 months	Time period 12 months	Time period 24 months
PD	Treatment	2.3	2.8	3	2.5
	Control	2.8	3.2	3.4	3.3
PAL	Treatment	1.8	2	2.1	0.5
	Control	2.2	2.2	2.3	2.6
DIB	Treatment	3	3	3.2	3
	Control	3	3.2	3.6	3.4

Mean peri-implant values were lower in treatment group during interval

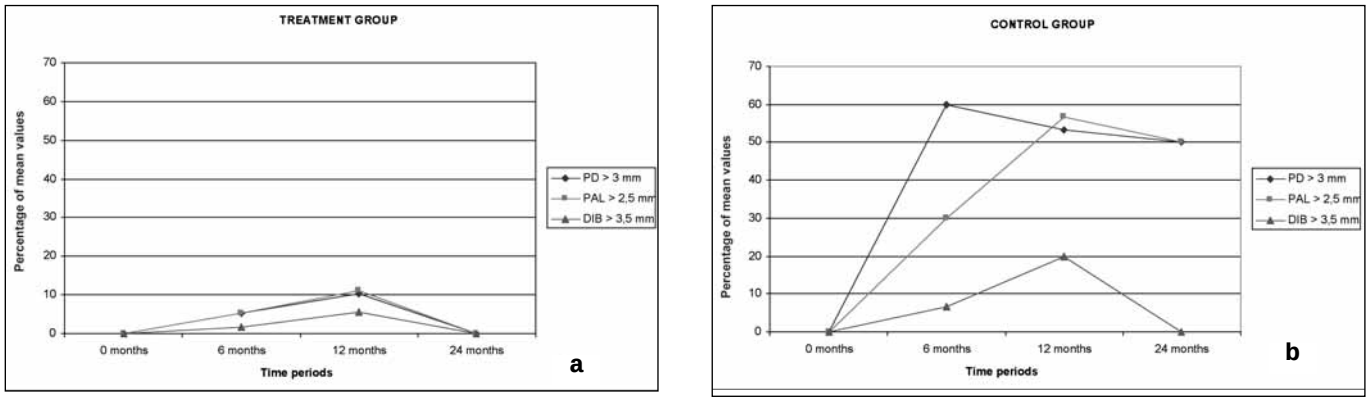

Fig. 5 - *Comparative analysis depicting the trend of peri-implant tissues in the treatment group (a) and the control group (b). Mean values were calculated with PAL >2.5 mm, PD >3 mm and DIB >3.5 mm. Mean scores attested more stable peri-implant status in treatment group, during 2 yrs of analysis.*

TABLE IV - COMPARATIVE ANALYSIS OF PERCENTAGE OF PERI-IMPLANT MEAN SCORES RECORDED WITH PD > 3 mm, PAL > 2.5 mm AND DIB > 3.5 mm

	Groups	Time period 0 months	Time period 6 months	Time period 12 months	Time period 24 months
Treatment	PD > 3 mm	0	5.2	10.3	0
	PAL > 2.5 mm	0	5.2	11.1	0
	DIB > 3.5 mm	0	1.7	5.6	0
Control	PD > 3 mm	0	60	53.3	50
	PAL > 2.5 mm	0	30	56.7	50
	DIB > 3.5 mm	0	6.7	20	0

More stable peri-implant status was attested in treatment group

tion of peri-implant tissues (Tab. IV).

The trend in PAL scores was similar to PD scores (Tab. III). For PAL scores it was also necessary to confirm the percentage of healthy sites. Therefore, the percentage of sites probed was calculated for each single interval group with values up to 2.5 mm (Tab. IV). The trend in this parameter appeared analogous to PD for scores up to 3 mm.

Biologically, the distance between DIB crest should be approximately 3.5 mm for the implants used in this study. A linear measure of DIB ≤3.5 mm was assumed as optimal. DIB scores >3.5 mm were then collected and processed for comparative analysis. The data collected in Table IV demonstrated the different trend in the values among the treatment group and the control group during the 1st yr. The percentages of DIB scores >3.5 mm were integrated with the percentage of PAL mean scores >2.5 mm and with PD scores >3 mm.

DISCUSSION AND CONCLUSIONS

In the past 25 yrs, numerous *in vivo* studies have demonstrated that non-submerged titanium implants achieve osseointegration as predictable as that of submerged titanium implants (4). This observation was confirmed in prospective clinical studies. The studies demonstrated success rates well above 90%. In summary, the non-submerged approach is a true alternative to the original healing modality with submerged titanium implants. The non-submerged approach offers several clinical advantages: 1) the avoidance of a second surgical procedure and less chair time per patient, resulting in overall reduced treatment cost; 2) the lack of microgap at the bone crest level, leading to less crestal bone during healing and resulting in a more favorable crown-to-implant length ratio; and 3) a simplified prosthetic procedure, presenting an ideal basis

for cemented implant restorations. Due to these significant clinical advantages, the non-submerged approach will become more important in implant dentistry in the near future, particularly in implant sites without esthetic priority. The creation of a new implant with a white transmucosal ceramic collar allowed the combination of the advantages offered by a transmucosal implant method with natural transparency at the transmucosal level widening applications in restorations in the anterior esthetic zone.

The advantages of transmucosal healing implants with a bioactive zirconia collar as a support of partially fixed prosthodontic restorations are optimal peri-implant marginal tissue sealing, reduction in plaque accumulation and satisfactory esthetic results. Zirconia is a widely studied material that has evidenced good mechanical performances (7) with reduced bacterial colonization (8) and reduced plaque accumulation (9). In addition, the zirconia used in this study evidenced not only optimal clinical performances but also good biocompatibility. *In vitro* tests demonstrated that zirconia coated titanium, compared to titanium, has a better compatibility related to the aspect considered. Results from this study show that zirconia coating enhances fibroblasts and, particularly, osteoblast-like cell adhesion, spreading and proliferation, favoring microscopic tissue / cell in growth and clinical implant fixation improvement.

From clinical analysis, it emerged that the treatment group obtained better scores in every peri-implant parameter. This evidence attests faster stabilization of soft and hard tissues around both the transmucosal zirconia collar and at the crestal level of the implant (Fig. 5a). The same parameters evaluated for implants with titanium collars demonstrated, instead, a major sensitivity of the peri-implant tissues to the modifications of the biological and biomechanical environment once the implant supporting rehabilitation started working (Fig. 5b). The association of factors such as plaque accumulation and load can perhaps play an important role in determining this tissue response. Reduced plaque accumulation around the implant with a zirconia collar could provide a better peri-implant microbiological environment by allowing the soft tissue expression of optimal sealing and good bone adaptation to loading. From these clinical and radiographic comparative analyzes, it emerged that in the treatment group the mean values were always similarly low. A rapid stabilization of both hard and soft peri-implant tissues was documented in the 1st yr. In the treatment group, there was the formation of stable tissue sealing the zirconia collar, which could preserve mucosal and bone levels.

In conclusion, 2-yr clinical results demonstrated that implants supporting fixed restorations using transmucosal healing implants with zirconia collars appeared as a valid method, reporting 100% implant survival rates. In addition, *in vivo* results obtained using strict parameters to assess the peri-implant status confirmed that a zirconia collar offers excellent biological acceptance according to our preliminary *in vitro* results, which evidenced statistically increased fibroblast and osteoblast adhesion and proliferation on zirconia coated titanium compared to the uncoated form, and an index of enhanced material integration with bone and soft tissue cells.

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