COMPARISON BETWEEN IMPLANT RETRIEVED FROM HUMAN MANDIBLE AND MAXILLA: AN HISTOMORPHIC-METRIC EVALUATION

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The aim of the present study was to compare the course of osseointegration speculating the bone-to-implant contact (BIC) rate, the osteocytes density (OD) and the collagen fiber orientation (CFO) in one implant retrieved from mandible and in one retrieved from maxilla. A SLA (Sand-blasted, Large grit, Acid-etched) surface implant of 3.3 mm x 15 mm was placed in a male 53 years old in the anterior region of the mandible bone (4.1) and an implant Dental Implant Line (sand blasted surface) of 3.75 x 16 mm was placed in the anterior region of the maxillary bone (2.1) in a female of 50 years old after a bone augmentation procedure. These implants were processed for histology. The specimens were analyzed under the confocal scanning laser microscope (CSLM) and brightfield light microscope (LM) equipped with circularly polarized light (CPL). The BIC rate of the implant retrieved after 23 months was 76.7± 4.9 (mean ±SD) while for the implant retrieved after 13 years it was 68.7 ± 3.7. The histomorphometric evaluation showed a predominantly woven bone around the 23 months implant, while around the 13 years implant the bone was manly lamellar. The transverse CFO (mean ±SD) under the lower flank of the thread near the tread tip was 55.2 ± 4.8 x 10^4 pixel for the 23 months specimen and 20.4 ± 3.5 x 10^4 for the 13 years specimen (P<.05). The longitudinal CFO (mean ±SD) in the inter-threads region was 65.6 ± 6.5 x 10^4 pixel for the 23 months specimen and 21.4 ± 3.0 x 10^4 for the 13 years specimen (P<.05). In the 23 months specimen much more longitudinal CFO were present in the inter-threads area, while under the lower flank of the thread there were much more transverse CFO. In the 13 years specimens the difference in transverse and longitudinal CFO appeared to be not statistically significant due to the lamellar nature of the peri-implant bone which presents alternating CFO in adjacent bone lamellae. The OD (mean ±SD) was 205 ± 45 in the specimen after 23 months and 130 ± 34 in the specimen after 13 years (P<.001).

Osseointegration is widely accepted in clinical dentistry as the basis for dental implant success. Failure to achieve osseointegration at a high rate can be attributed to one or more implant, local anatomic, local biologic, systemic or functional factors (1). Moreover, the performance and maintenance of osseointegrated dental implants in function are dependent upon several factors: 1) load transmission at the bone-to-implant interface (2); 2) the amount and quality of the bone (3); and 3) the surface characteristics of the implant (4).

A major interest in implant design factors is evident and clinical efforts to improve implant success have been focused on increasing the amount of bone that forms at the endosseous implant surface. Implant surface character is one implant design factor affecting the rate and extent of osseointegration .Following the osseointegration the bone tissue is formed around an alloplastic material (implant) in an hierarchical mode (following at the best the implant macro-micro-nano-structures) satisfying the environmental needs (unloading vs. loading) with stiffness and adequate strength. Yet to maintain the osseointegration bone must be adaptable and repairable.

Key Words: Circularly Polarized Light Microscopy; collagen fiber orientation; bone implant contact rat; implant fracture; osseointegration.

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So the peri-implant bone architecture is an answer to the above requirements.

Until now, most of the histological analyses in implant dentistry were based on the bone to- implant contact (BIC) without also considering bone matrix organization. However, the mechanical properties of the bone and its spatial arrangement are considered to be very important factors (5). Considerable amount of experimental and numerical studies have been performed on understanding the mechanism of load transfer from the implant to the bone using the finite element analysis (FEA) methods. Nevertheless, some limitation of FEA methods still remain i.e. the anisotropic character of the bone tissue, the lack of spatiotemporal dynamic response of the bone cells and consequently bone matrix rearranging. The peri-implant bone adjusts its architecture in relation to its functional load bearing (6). The differences between implant surfaces and morphologies are important for the stability of implants under load and are also of major importance for the bone forming osteoblasts to promote matrix mineralization in the vicinity of the implant. Primary bone is easily distinguished from secondary osteons of remodeled bone since, secondary osteons are formed through a resorption and replacement process and their outer margin will often intersect lamellae of the surrounding bone. Additionally, secondary osteons can usually be distinguished from primary osteons by their reoriented collagen matrix, as seen under Circularly Polarized Light Microscopy (CPLM), and their circumferential margin known as the cement line. By definition, primary osteons do not have cement lines.

The principal factor in determining the mechanical properties of bone is the collagen configuration in the matrix and corresponding orientation of mineral crystallites that also reflects the mechanical microenvironment at the time of bone formation (7). So, the quantity and orientation of the collagen fibers surrounding the implant can serve as a reliable measure of osseointegration quality. Determining the collagen fiber orientation (CFO) in peri-implant bone matrix by a means of CPLM is an alternative avenue to study the load transfer along the bone-dental implant interface over the time. The different mechanical loading environment affects the collagen orientation and it could alter osteoblasts in a manner that causes a characteristic orientation in the fibers they synthesize: bone under compression shows transversely oriented collagen, whereas bone under tension shows longitudinally oriented collagen (8). Moreover, the CFO in bone could help obtain information about the relation among implant design, distribution of stress applied to the bone, and the growth of bone (9).

Given recent findings implicating osteocytes as regulators of bone remodeling, bone formation and bone volume (10). Osteocytes are the most abundant cells in the bone that form a cellular syncytium able to sense the local environment and to influence bone remodeling (11). Moreover, they play a crucial role in maintaining the mechanical quality of bone: osteocyte density is positively related to the proportion of osteoid surface covered by osteoblasts and it could be considered as an alternative index in assessing bone quality.

The aim of the present study was to compare the course of osseointegration speculating the bone-to-implant contact (BIC) rate, the osteocytes density and the collagen fiber organization in one implant retrieved from mandible and one retrieved from maxilla .

MATERIALS AND METHODS

Implants

A SLA (Sand-blasted, Large grit, Acid-etched) surface implant (Arrow Press fixture, Alpha Bio LTD, Petah-Tikva, Israel) of 3.3 mm x 15 mm was placed in a male 53 years old in the anterior region of the mandible bone (4.1) in June 2008. The implant was immediately loaded with an acrylic resin restoration. Final restoration in glass-ceramic fused to zirconia was placed two months later. The implant-restoration undergo to fracture in July 2010 (after 23 months).

In a female of 50 years old, an implant Dental Implant Line (sand blasted surface) (Dental Implant Line, Casalpalocco, Roma, Italy) of 3.75 x 16 mm placed in the anterior region of the maxillary bone (2.1) after a bone augmentation procedure made in January 1997 using either DFDBA autologous bone chips and Gore Tex membrane was used. The implant, placed four months later the augmentation procedure, remained in function until to July 2010 (13 years) when it was removed due to implant platform fracture.

Specimen's processing

The retrieved specimens were fixed in 4% formalin pH 7.0 for 10 days, and then transferred to a solution of 70% ethanol until processing. The specimens were dehydrated in increasing concentrations of alcohol up to 100%, infiltrated and embedded in LR White (London Resin Company, Berkshire, England) resin. Undecalcified longitudinal cut sections of 50 µm were prepared by using a cutting and grinding TT system (TMA2, Grottammare, AP, Italy). The sections were double stained with toluidine blue and fuchsine acid for some samples and toluidine blue stains for others to be analyzed.

Transmitted Light Microscopy (LM)

The histomorphometry was used to evaluate the amount of bone implant contact rate (BIC%). The investigation was carried out in a transmitted brightfield Light Microscope Axiolab (Zeiss Oberchen, Germany) connected to a high-resolution digital camera (FinePix S2 Pro, Fuji Photo Film Co. LTD. Minato-Ku, Japan). An Histometric software package with image capturing capabilities (Image-Pro Plus 6.0, Media Cybernetics Inc., Bethesda, MD, USA) was used. To ensure accuracy, the software was calibrated for each experimental image using a software feature named "Calibration Wizard" which reports the number of pixel between two selected points (diameter or length of the implant). The linear remapping of the pixel numbers was used to calibrate the distance in millimeters

Circularly Polarized Light Microscopy (CPLM)

Birefringence was used to evaluate the collagen fiber orientation (CFO) of the bone matrix around the implants. The measurements using polarized light were concentrated mainly under the thread tip along the lower flank of the thread and in the inter-threads region. Unstained sections (before staining procedure) were used. The CFO was evaluated by a means of a light microscope (Axiolab, Carl Zeiss, Jena, Germany) equipped with two linear polarizer and two quarter wave plates arranged to have transmitted circularly polarized light, connected to a high-resolution digital camera (FinePix S2 Pro, Fuji Photo Film Co. LTD. Minato-Ku, Japan). The Collagen fibers aligned perfectly transverse to the direction of the light propagation (parallel to the plane of the section) appeared "white-blue" due to a change in the refraction of exiting light whereas the collagen fibers aligned along the axis of light propagation (perpendicular to the plane of the section) appeared "red-yellow", because no refraction occurred.

Confocal Scanning Laser microscopy

In order to evaluate the osteocytes/ lacunae density the specimens were stained using basic fuchsin, than evaluated under a confocal scanning laser microscope (CSLM), TCS-SP, Leica Microsystems, Wetzlar, Germany) with a 20 x magnification objective lens. A 568 nm wavelength excitation light was used to view the fluorescent die. The digitized images were stored in format JPEG with NxM = 3024 x 2016 grid of pixels for a 24 bit. The osteocytes density (OD) was evaluated as follow OD = Ot.Lc/BAr where Ot.Lc was the number of osteocytes or lacunae counted while BAr was the bone area investigated.

Statistical analysis

One person (TT) performed all the measurements. Intra-examiner variability was controlled by carrying out 2 measurements for each index. When for the same index the difference in the two performed readings exceeded 15 % the measure was repeated. Statistical analysis was performed by means of the computerized statistical package (Sigma Stat 3.5, SPSS inc. Ekrath, Germany). To compare the BIC rate for a significant difference Z-test was used between two implants. Parametric tests were used to test the histomorphometric results after evaluating both the normality test and the equal variance test. Unpaired t-test was used in the inference of the OD between two groups while, One-Way ANOVA test was used to evaluate the CFO toward either implant type and implant site followed by a multiple comparison procedure using Holm-Sidak method. A P value of under 0.05 was considered statistically significant.

RESULTS

The BIC rate of the implant retrieved after 23 months was 76.7± 4.9 (mean ±SD) while for the implant retrieved after 13 years it was 68.7 ± 3.7 (Fig. 1). The difference was not statistically significant (P= 0.632) (Fig. 2, Table I). The histomorphometric evaluation showed a predominantly woven bone around the 23 months implant, while around the 13 years implant the bone was manly lamellar. The transverse CFO (mean ±SD) under the lower flank of the thread near the tread tip was $55.2 \pm 4.8 \times 10^4$ pixel for the 23 months specimen and $20.4 \pm 3.5 \times 10^4$ for the 13 years specimen (P<.05) (Fig. 3, Table II) . The longitudinal CFO (mean ±SD) in the inter-threads region was $65.6 \pm 6.5 \times 10^4$ pixel for the 23 months specimen and $21.4 \pm 3.0 \times 10^4$ for the 13 years specimen (P<.05) (Fig. 3, Table II). In the 23 months specimen much more longitudinal CFO were present in the inter-threads area, while under the lower flank of the thread there were much more transverse CFO (Fig. 4). In the 13 years specimens the difference in transverse and longitudinal CFO appeared to be not statistically significant due to the lamellar nature of the peri-implant bone which presents alternating CFO in adjacent bone lamellae. The OD (mean ±SD) was 205 ± 45 in the specimen after 23 months and 130 ± 34 in the specimen after 13 years (P<.001) (Fig. 5 and 6, Table III).

DISCUSSION

The clinical use of available osseointegrated titanium implants to substitute missing teeth is widespread notwithstanding our knowledge of the processes which take place on the interface during healing is limited. Moreover, we do not know how several types of implants become attached to the bone that is the processes involved in the mechanical interconnections compared to the chemical one. This lack of information is possibly due to the lack of techniques for the specific study of these problems.

However, the osseointegration is widely accepted in clinical dentistry as the basis for dental implant success and first this requires a peri-implant bone tissue of good quality. Failure to achieve osseointegration at a high rate can be attributed to one or more implant, local anatomic, local biologic, systemic or functional factors (1).

Implant surface character, an important implant design factor, represents a great importance for the implant success because affecting the rate and extent of osseointegration. For this reasons clinical efforts have been focused on increasing the amount of bone that forms at the endosseous implant. During the process of the osseointegration, after dental implant placement, the bone tissue is formed around an alloplastic material (implant) in an hierarchical mode (following at the best the implant macro-micro-nano-structures) satisfying the environmental needs with stiffness and adequate strength. One important factor that influences the formation of the

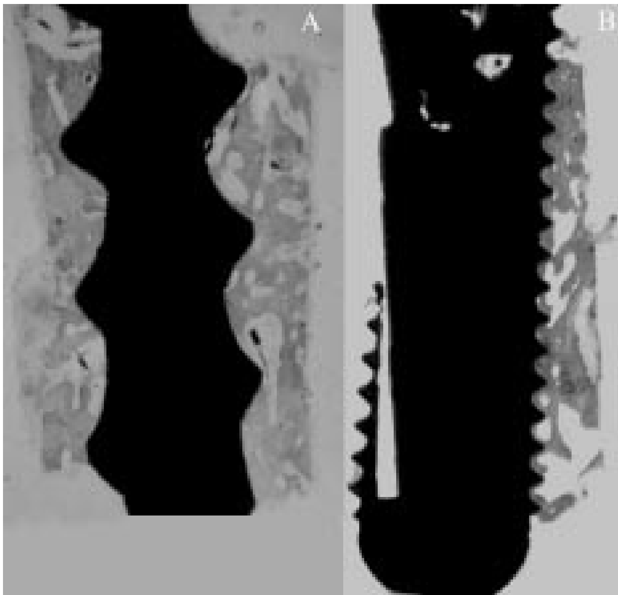

Fig. 1. *Light microscopic view of bone implant contact. In (A) immediate loaded implant retrieved due to fracture (overloaded) after 23 months. In (B) delay loaded implant retrieved after 13 years due to implant platform fracture. Toluidine blue staining; original magnification x 12*

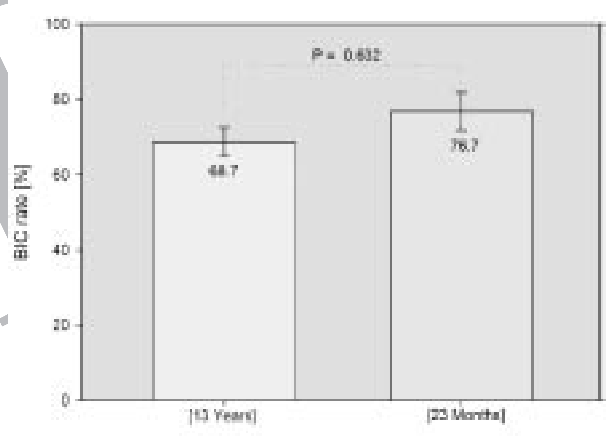

Fig. 2. *BIC rate comparison by time. The difference in the mean values of the two groups appeared to be not statistically significant (P= 0.632).*

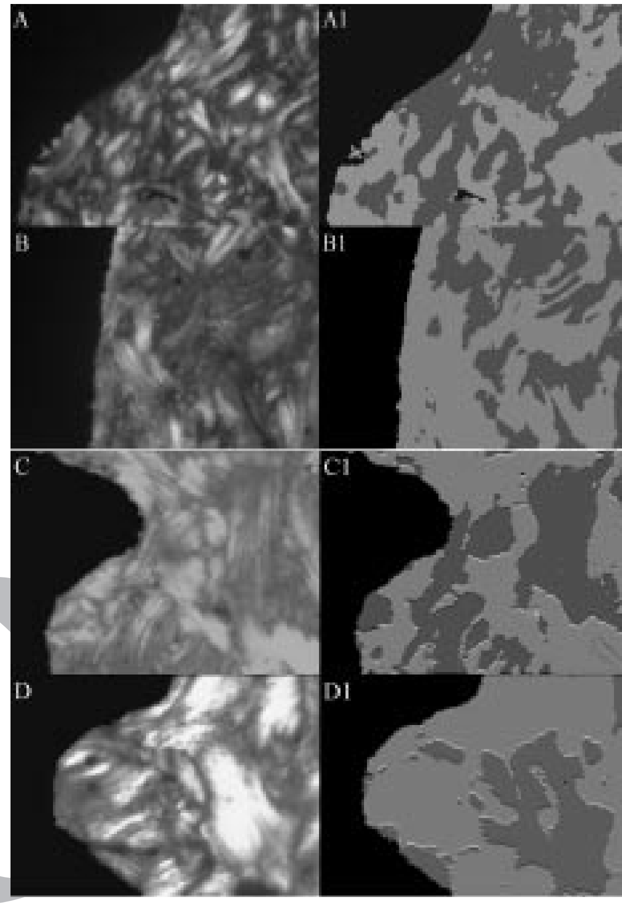

Fig. 3. *Polarized light microscopic images of the bone near the implant retrieved after 23 months (A ; B) and after 13 years (C;D). in (A) the bone facing the lower flank of the thread (near the tip), where the load was transferred to bone by compressive vectors, the CFO appeared mainly transverse (white-blue)than longitudinal (white-red). In (A1) the computer separation of the two CFO orientation. In (B) the bone facing the inter-threads, were the load was transferred to bone mainly by shear vectors, the CFO was mostly longitudinal (white-red). In (B1) the computer separation of the two CFO orientation. In (C) the bone facing the lower flank of the thread the CFO appeared mainly transverse (white-blue). In (C1) the computer separation of the two CFO orientation. In (D) the bone facing the inter-threads the CFO was mostly longitudinal (white-yellow). In (D1) the computer separation of the two CFO orientation. Unstained sections; original magnification x 100.*

bone-to-implant contact (BIC) is the mechanical loading that sometimes is applied immediately after implant placement. In fact, immediate loading has recently become one of the most important research topics in restorative dentistry and has as main objective, to achieve a high mechanical stability to avoid micromovements during the process of osseointegration (12). Yet to maintain the osseointegration bone must be adaptable and repairable. So, the knowledge of the peri-implant bone architecture is an answer to the above requirements.

Implants in function among others, undergo to axial load that exerts a considerable lateral forces which can be estimated to F tan (α) / (2πD) where F, vertical load; D, is the diameter of the implant. All the occlusal forces

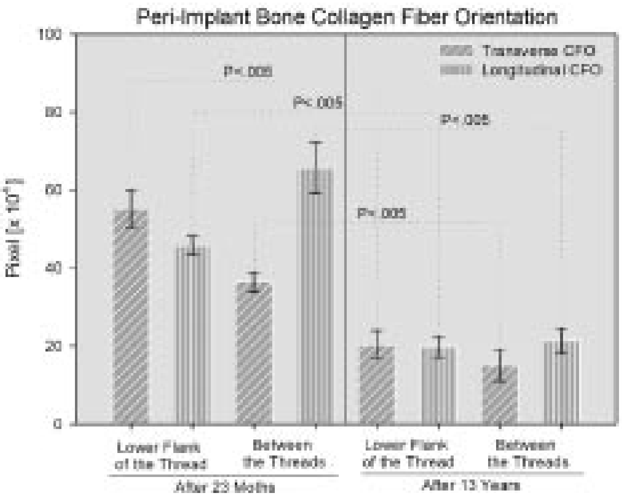

Fig. 4. *The CFO comparison for transverse and longitudinal collagen fiber vs 23 months and 13 years by lower flank of the thread or between threads. It was noted a statistical significant association for both implants for transverse CFO to the lower flank of the thread and for longitudinal CFO to the inter-threads area. In general the implant retrieved after 23 months showed much more difference in CFO due to the presence of woven bone than the implants retrieved after 12 years in which the bone appeared to be lamellar.*

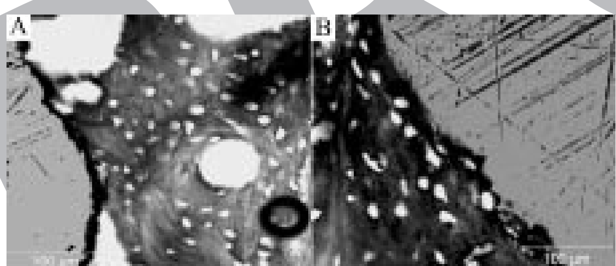

Fig. 5. *Confocal laser micrographs of the bone around dental implants after both 23 months (A) and 13 Years(B). The implant appears in gray, the mineralized bone matrix appears in black while the osteocytes appears in yellow. Basic fuchsine staining; original magnification x 200.*

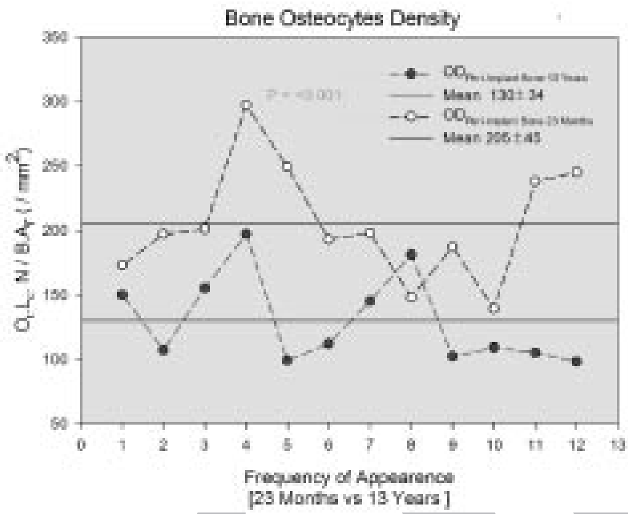

Fig. 6. *The osteocyte lacunar density ($O_tL_c.N/BA_t$) comparison between peri-implant bone after either 23 months and 13 years. The density is significantly higher after 23 months than that 13 years (P<.001).*

are transmitted directly to the bone around the implant so, the distribution at the implant-bone interface is influenced by the implant design. The load has a profound effect on the organization of the bone matrix composition and organization.

Bone is a two-phase porous composite material constituted primarily of collagen and minerals, which together provide its mechanical properties (13). The process of osseointegration culminates in calcium phosphate crystal growth on matrix proteins like osteopontin and bone sialoprotein and collagen production with mineralization (14).

Furthermore, the important and complex interactions of extracellular matrix macromolecules like collagen fibers with mineral deposits under mechanical loads are still poorly known. While the mineral phase primarily imparts stiffness to bone, the spatial orientation of collagen fibers contributes to bone toughness and strength (mechanical properties) (15).

Classical histology classified bone tissue in relation to the spatial orientation of collagen fibers. Two several types of bone have been recognized: woven-fibered bone and parallel-fibered bone (lamellar or non-lamellar). Woven bone has a loosely organized matrix that is formed rapidly in response to fracture healing or hypertrophic adaptation. The slower parallel-fibered bone displays a more highly organized matrix and greater strength. The stiffness and strength of the bone is also related to the degree of matrix mineralization (16).

The hypothesis that collagen fibers of preferred orientation and distribution have biomechanical significance was first set forth by Gebhardt (17) and later tested by Ascenzi and Bonucci (18) and Simkin and Robin (19). Numerous studies correlate strongly the collagen fiber orientation to the mechanical loading (20). However few studies have, until now, focused on the relationship between collagen fiber orientation and loaded dental implants although the orientation of the collagen fibers enable evaluation of the quality of osseointegration and bone remodeling (21) The orientation of collagen fibers in bone could help obtain information about the relation among implant design, distribution of stress applied to the bone, and the growth of bone (9).

Table I. *BIC rate comparison.*

Z-test		
Group Name	**N**	**Proportions**
13 Years	5	0.680
23 Months	5	0.760
Yates correction applied to calculations		
The difference of sample proportions		-0.0800
The pooled estimate for p= 0.716		
Standard error of difference of sample proportions= 0.303		
95 percent confidence interval for difference: -0.673 to 0.513		
z= -0.479; P = 0.632		

Table II. *Longitudinal / transversal CFO vs implant site multicomparisons*

One-Way ANOVA for CFO					
Normality Test: Passed (P = 0.178)					
Equal Variance Test: Passed (P = 0.927)					
Group Name		**N**	**Mean [pixel x10^4]**	**Std Dev**	**SEM**
Transverse CFO Thread Tip 23months [A]		5	55.200	4.817	2.154
Longitudinal CFO Tread Tip 23months [B]		5	45.800	2.387	1.068
Transverse CFO Inter-Treads 23months [C]		5	36.400	2.408	1.077
Longitudinal CFO Inter-Threads 23months [D]		5	65.600	6.542	2.926
Transverse CFO Thread Tip 13years [E]		5	20.400	3.578	1.600
Longitudinal CFO Thread Tip 13years [F]		5	19.800	2.864	1.281
Transverse CFO Inter-Threads 13years [G]		5	15.000	4.000	1.789
Longitudinal CFO Inter-Threads 13years [H]		5	21.400	3.050	1.364
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	7	12460.700	1780.100	115.031	<0.001
Residual	32	495.200	15.475		
Total	39	12955.900			
The differences in the mean values among the groups are statistically significant (P = <0.001).					
Power of performed test with alpha = 0.050: 1.000					
All Pairwise Multiple Comparison Procedures (Holm-Sidak method): Overall significance level = 0.05					
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**	**Significance**
[A] vs. [E]	34.800	13.987	3.492E-015	0.002	yes
[B] vs. [F]	26.000	10.450	7.634E-012	0.003	yes
[C] vs. [G]	21.400	8.601	0.000000000789	0.003	yes
[D] vs. [H]	44.200	17.765	3.895E-018	0.002	yes

Table III. *OD comparison.*

Unpaired t-test for OD				
Normality Test: Passed (P = 0.075) Equal Variance Test: Passed (P = 0.630)				
Group Name	N	Mean	Std Dev	SEM
OD peri-imlant bone 23 mounths	12	130.000	34.415	9.935
OD peri-implant bone 13 years	12	205.417	45.038	13.001
Difference -75.417				
t = -4.609 with 22 degrees of freedom. (P = <0.001)				
95 percent confidence interval for difference of means: -109.351 to -41.483				
The difference in the mean values between the groups are statistically significant (P = <0.001). Power of performed test with alpha = 0.050: 0.995				

In the present study the much more amount of either transverse or longitudinal CFO in the 23 months specimen are explained by both a higher BIC rate and the presence of woven bone. Moreover for the 13 years specimen the results showed mainly lamellar bone with several secondary "hoop" alternating osteons with transverse and longitudinal CFO. This aspect, heavily contributes to a lack of a predominant CFO in the 13 years specimen. The analysis for transverse and longitudinal CFO for different implant sites point out the importance of the implant shape factor. In fact, both implant specimens' showed differences for bone CFO when we comparing the thread tip area vs inter-thread area demonstrating differences in loading transfer. Moreover, under the lower flank of the thread the prevalence of transverse CFO is an index of compression stress while in the inter-threads region the prevalence of longitudinal CFO indicates the presence of a shear stress. The different thread profile for the two type of implants here investigated appear to be of importance for bone matrix composition around loaded dental implants. This aspect will be further investigated.

Beside the spatial orientation of collagen fibers, also bone cells play a determinant role in achieving a satisfying implant success. After dental implant placement, the healing process forms a soft fracture callus with a conspicuous neoangiogenesis, supporting the precursor bone cells. Frost (22) assumed the `basic multicellular units' (BMUs) of osteoblasts and osteoclasts come the local strains to maintain local bone mass (mechanostat theory). They are controlled by a `mechanical feedback loop' and a `set point', which is the quantitative setting for the balance between strain and bone mass. The same Author pointed out that this control process is purely biological in its components, but is governed by mechanical loads. It is known that static loads have no effects on bone mass; experimental information makes it plausible that amplitude, rate, frequency and duration of loading are all important for bone metabolism. These theories have contributed greatly to the awareness of mechanical factors in the regulation of bone modeling, remodeling and repair in orthopedics, orthodontics and in general in bone biology.

Initially, osteoblasts formed woven bone and when they ended bone matrix synthesis, either dead by apoptosis or differencing into osteocytes embedded in the matrix or remaining on the surface becoming lining cells (23).

Osteocytes, the most abundant cells in the mature bone, are the ideal location to form a cellular syncytium able to sense the local mechanical environment and to influence bone remodeling (11). Osteocyte density is positively related to the quantity of osteoid surface covered by osteoblasts and would be inversely related to the proportion of osteoblasts that undergo apoptosis. Moreover, some authors have been reported that osteocytes might also be more numerous in bone with higher turn-over (24).

The osteocyte syncytium within bone is responsible for sensing load and regulating functional bone adaptation via the canalicular network and intercellular gap junctions working as the mechanosensors (11). Recently, Ma YL et al.(25) suggested that osteocytes plays a crucial role in maintaining the biomechanical quality of bone, and osteocyte density could be considered as an alternative index in assessing bone quality.

In conclusion the bone around the implant retrieved after 23 months appeared to be woven with a significantly increase of osteocytes number while the bone around the implant retrieved after 13 years was mainly lamellar with several secondary osteons. For both implants the transverse CFO were associated with the lower flank of the implant threads, while the longitudinal collagen fibers were more represented in the inter-threads area.

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