JOURNAL OF APPLIED ORAL SCIENCE
www.fob.usp.br/revista or www.scielo.br/jaos
J Appl Oral Sci *2005; 13(1): 87-92*

EVALUATION OF AN IBAD THIN-FILM PROCESS AS AN ALTENATIVE METHOD FOR SURFACE INCORPORATION OF BIOCERAMICS ON DENTAL IMPLANTS. A STUDY IN DOGS

AVALIAÇÃO DE UM RECOBRIMENTO DE BAIXA ESPESSURA PROCESSADO PELA DEPOSIÇÃO ASSISTIDA POR FEIXE IÔNICO COMO ALTERNATIVA PARA A INCORPORAÇÃO DE BIOCERÂMICAS EM IMPLANTES DENTÁRIOS. ESTUDO EM CÃES

Paulo G. COELHO[1], Marcelo SUZUKI[2]

1- B.S., Engineer, Microgravity Solidification Laboratory, Dept. of Materials Science and Engineering, University of Alabama at Birmingham School of Engineering
2- D.D.S., M.S., Graduate Prosthodontics Resident, Dept. of Prosthodontics and Biomaterials University of Alabama at Birmingham School of Dentistry

Corresponding address: Paulo G. Coelho - Engineer, Microgravity Solidification Laboratory, Dept. of Materials Science and Engineering, University of Alabama at Birmingham School of Engineering - 1150 10th ave. south, BEC 254 - Birmingham, AL 34461
E-mail: paulocoelho@bellsouth.net - (205) 934-0878 - (205) 934-8485 fax

Received: September 9, 2004 - Modification: November 22, 2004 - Accepted: January 06, 2005

ABSTRACT

Thin-film bioceramic coatings are potential alternatives to overcome the limitations provided by other commercially available coating techniques like PSHA, where variable bioceramic dissolution added to a metalloceramic weak link are process- inherent. The purpose of this investigation was to determine the overall and site specific (to 0.5 mm from implant surface) levels of osseoactivity around a thin-film (IBAD processed) coated titanium alloy implant versus a non surface modified (sand-blasted/acid etched) titanium alloy implant in a canine model. The surgical model comprised the proximal tibiae epiphyses with four implants placed in each limb remaining for 2 and 4 weeks *in-vivo*. 10 mg/Kg oxytetracycline was administered 48 hours prior to euthanization. The limbs were retrieved by sharp dissection, reduced to blocks, and subsequently nondecalcified processed for fluorescent microscopy. Micrographs (20x mag) were acquired around the implant perimeter and merged for overall biological response evaluations, and four micrographs (40x mag. subdivided in rectangles) were acquired along one of the implant sides for tetracycline labeled area fraction quantification. The results showed biocompatible and osseoconductive properties for the thin-film coated and uncoated titanium alloy implants. Tetracycline labeled area fraction analyses showed that the thin-film coated implants presented significantly higher overall and site specific osseoactivity levels at 2 and 4 weeks. The site specific osseoactivity values were significantly higher compared to overall values for control and thin-film coated implants at both times *in-vivo*. According to the results obtained in this study, thin-film coated implants enhanced biological response at the early implantation times evaluated.

Uniterms: Dental implants; Thin-film coatings; Osseointegration; Tetracycline labeling; Bioceramic; Histomorphometry measurements; Dogs.

RESUMO

Recobrimentos biocerâmicos de baixa espessura são potenciais alternativas para compensar as limitações de outros recobrimentos biocerâmicos disponíveis comercialmente como o plasma spray de hidroxiapatita, onde a dissolução desigual e a presença de uma fraca interface metal-cerâmica são problemas inerentes ao seu processamento. O propósito desta investigação foi determinar os níveis de atividade óssea total e específica a uma área (0.5 mm da superfície do implante) ao redor de um implante de liga de titânio (superficie jateada seguida de ataque ácido) recoberto com um filme biocerâmico de baixa espessura processado através de deposição auxiliada por feixe iônico, contra um implante de liga de titânio sem recobrimento biocerâmico em cães. O modelo cirúrgico utilizou a epífise proximal da tibia, com quatro implantes colocados em cada uma, onde permaneceram por um período de 2 e 4 semanas. Oxitetraciclina (10 mg/Kg) foi administrada 48 horas antes dos animais serem sacrificados. As tíbias foram dissecadas, reduzidas a blocos, e processadas para análise em microscópio ótico. Microfotografias com aumento de 20x foram obtidas da região perimetral do implante e foram alinhadas para análise da resposta biológica total. Subsequentemente, quatro micro-fotografias com aumento de 40x, sub- divididas em retângulos, foram obtidas de um dos lados do implante para quantificação da área marcada por tetraciclina. Os resultados mostraram biocompatibilidade e osseocondutividade dos implantes de liga de titânio com ou sem filme biocerâmico de baixa espessura. Análise da área marcada por tetraciclina mostrou que os implantes com recobrimento apresentaram uma maior atividade óssea total e específica ao redor do implante em 2 e 4 semanas. Os valores de atividade óssea específica à área adjacente à superfície do implante foram significantemente maiores comparados aos valores obtidos em regiões afastadas dos implantes com ou sem recobrimento biocerâmico. De acordo com os resultados obtidos neste estudo, concluímos que os implantes com recobrimento biocerâmico de baixa espessura aumentaram a resposta biológica após 2 e 4 semanas de tempo de implantação.

Unitermos: Implantes dentários; Recobrimentos de baixa espessura; Osseointegração; Tetraciclina; Análise histomorfométrica; Biocerâmica; Cães.

INTRODUCTION AND BACKGROUND

The success of dental implants requires their anchorage in bone in order to withstand functional loading. This idea is accepted since both archaeology and histology records provide evidence of dental implants endosseous integration[5].

Significant evolution on both surgical and restorative aspects of dental implantology have occurred since the term osseointegration[2] (direct bone apposition at the surface of either titanium or titanium alloys) was defined by a Swedish research group in the late seventies[1]. This term has been constantly redefined and by no means represents the complexity of the phenomena occurring at the bone-biomaterial interface.

While predictable outcomes have been reported since endosseous' implants early days following the classical two-stage technique protocol, a desire for treatment time decrease while maintaining success rates reported above 90%[2-3] has been demonstrated by practitioners and patients.

Many attempts have been made on the manufacturing processes of dental implants in order to improve biological response of the host to materials. For this purpose, surface engineering methods have been under constant investigation, once it was known that some surface modifications notably changed the *in-vivo* performance of biomaterials[7,21,28].

Several engineering processes have been used to modify the surface of dental implants in an attempt to increase bone wound healing kinetics and decrease treatment time frames[20,22]. Among popular surface modifications are the incorporation of calcium- and phosphate-based bioceramics to the surface of commercially pure titanium and titanium alloys in the form of apatites[29] or phases of other stoichiometry (calcium to phosphate ratios)[20]. The elemental components of these phases are found in the composition of natural bone, leading to a rationale for employment of biomaterials synthetically manufactured to resemble these compositions as implant materials[20,22,29].

Plasma Spraying of Hydroxyapatite (PSHA) is by far the most commonly used coating technique for bioceramic incorporation on dental implants to the present day due to its processing versatility and simplicity, where virtually all implant bulk designs may receive a continuous coating layer on its surface[19-20,22]. Its manufacturing process has been thoroughly described[19], and PSHA coatings have been shown to elicit earlier biological responses around implants[8-9,11,17-18]. Limitations concerning PSHA processed bioceramic coatings are the variable dissolution rates due to the inherent multiphase microstructure obtained through this process[15,19-20,25,30], added to the presence of a metalloceramic weak link between the bulk metallic substrate and bioceramic coating, which relies on mechanical interlocking for its integrity and maintenance[20,22].

In an attempt to overcome the limitations of the PSHA process while still benefiting from the increased osseoconductive properties provided by bioceramic coatings, thin-films of highly controlled microstructures and thicknesses have been engineered on the surface of dental implants. These thin-films may be deposited by a variety of techniques including sol-gel, Pulsed Laser Deposition (PLD), Ion Beam Assisted Deposition (IBAD), and others[20,22]. A potential advantage of thin-film processes is the tailored bioceramic dissolution as a function of time *in-vivo*, providing implant surface exposure as implantation time elapses, enabling direct bone contact to the implant surface. This direct bone contact to the metallic substrate favors the bone-biomaterial interface mechanical competence by avoiding metalloceramic weak links between the metallic substrate and coating, as found on PSHA coated implants[20,22].

Due to the dynamic modeling/remodeling nature of bone during wound healing and homeostasis, there is a need for specific labeling tools for hard tissue kinetics' histomorphometric assessment. A bone tissue marker is defined as any identifiable feature, naturally occurring or artificially induced, which permits the location of a given bone surface in anatomical space at a known moment in time[26]. The use of tetracycline (TC) as a tissue marker was introduced[23-24], reviewed[4,13] and has been recently applied[6.16] due to its fluorescent properties, which allow for determination of osteoblastic activity (osseoactivity) in nondecalcified specimens. TC use has been extensive as a bone research tool regarding location and kinetics of bone formation and growth. This methodology has been utilized to evaluate overall and site specific bone activity levels around dental implants in various *in-vivo* models[10,14,27], including dogs and humans. These studies[10,14,27] indicated that the modeling/remodeling kinetics at regions adjacent to the implant surface (to approximately 1 mm from surface) may have significant differences compared to regions away from the surface at various times *in-vivo*, and it has been hypothesized[15] that short and long term stability of dental implants are related to this region of increased bone activity.

The purpose of this investigation was to determine the overall and site specific (to 0.5 mm from implant surface) levels of osseoactivity around a thin-film Ca- and P- based bioceramic coated titanium alloy (Ti-6Al-4V) implant manufactured by the IBAD process versus a non surface modified (sand-blasted/acid etched) titanium alloy implant by means of stereological techniques[12] (quantitative microscopy) in a laboratory dog model.

MATERIALS AND METHODS

Materials

The as-processed, sterilized, and packaged sand-blasted/acid-etched titanium alloy and thin-film coated titanium alloy implant rods were provided by the manufacturer (Bicon, Inc. Boston, MA-USA). These were 10 mm in length by 4 mm in diameter. The number of devices was 32 and included an experimental (thin-film bioceramic coated, n=16) and a control group (sand-blasted/acid-etched, n=16). No detail regarding surface topography and chemistry was provided by the manufacturer.

Methods

Surgical Model and Clinical Aspects

The surgical model comprised 4 mid-size class A adult (closed bone growth plates) mongrel dogs in good health. The dogs followed a 2-week housing period before the first surgical procedure and 4 weeks post-operatively. The project was conducted after IRB approval in an AALAC approved facility.

The surgical site was the proximal tibiae epiphyses, with four implants placed in each limb. Each dog provided a 2- and 4-week comparison between experimental and control surfaces *per* four-implant location through sequenced surgical procedures. The left limb was used for the 4-week evaluation and the right limb for the 2-week evaluation. The surgeries were conducted under full anesthesia following sterile methodologies.

Surgical Site Preparation and Implantation

The proximal tibiae were exposed subperiostally and 4 equi-spaced holes were drilled through sequential burs (under external saline irrigation). The implants were then inserted into the trabecular mid-region with its top in contact with the tibiae proximal cortical plate. A polymeric cover screw was threaded into the implant top and standard layered procedures were employed for soft tissue closure. Forty-eight hours prior to euthanization, 10 mg/kg oxytetracycline was administered subcutaneously to provide fluorescent labeling for histomorphometric analyses (single label).

Specimen Preparation

At necropsy, the proximal tibia was exposed by sharp dissection. The upper one half of the bone was removed and contact radiographed to confirm implant location and orientation. The limbs were reduced to blocks with the implant in its center, which were subsequently processed to thin sections approximately 20 μm in thickness with the metallic implant in place through standard procedures for optical light microscopy.

Hystomorphometric Analyses

General Biocompatibility Evaluation

The nondecalcified specimens were placed under an optical microscope equipped with an ultra violet source at 20x magnification, and 12 micrographs were acquired around the implant perimeter. The micrographs were merged by a computer software (Adobe Photoshop, San Jose, CA- USA), and the implant perimeter and surrounding bone structure was obtained for analysis. Qualitative evaluation regarding biocompatibility was performed for the different groups at both evaluation times *in-vivo*.

Tetracycline Labeling Quantification

Quantification of the tetracycline labeled bone area fraction was performed by acquiring 4 micrographs (40x magnification) along one side of the implant (total implant length covered at this magnification). Each of the four micrographs was subdivided into rectangles (0.5 mm base and 2.5 mm height) comprising 0.5 mm steps from the implant surface (Figure 1), and a 9-point grid was randomly placed 6 times for each micrograph subdivision for point-count[12] stereologic inferences. This procedure implied 24 tetracycline labeled bone area fraction measurements for each micrograph and a total of 96 measurements per implant.

The overall quantification of tetracycline labeled bone area fraction was assessed by considering all measured quantities for the 4 micrographs and their respective subdivisions for statistical analysis.

Investigation of the tetracycline labeled bone area fraction at the site in close proximity to the implant surface (to 0.5 mm from implant surface) was performed by only considering measurements obtained from the subdivision adjacent to the implant surface for the 4 micrographs acquired for statistical analysis.

Statistical Analyses

The confidence interval (CI) for each parameter evaluated through the quantitative microscopy technique described above was calculated at the 95% level of significance through the following equations: CI= [mean value ± t (standard error)], standard error = [standard deviation/($n^{1/2}$)], where t= t value associated with the number of degrees of freedom and level of significance, and n= number of observations for the parameter under evaluation[12].

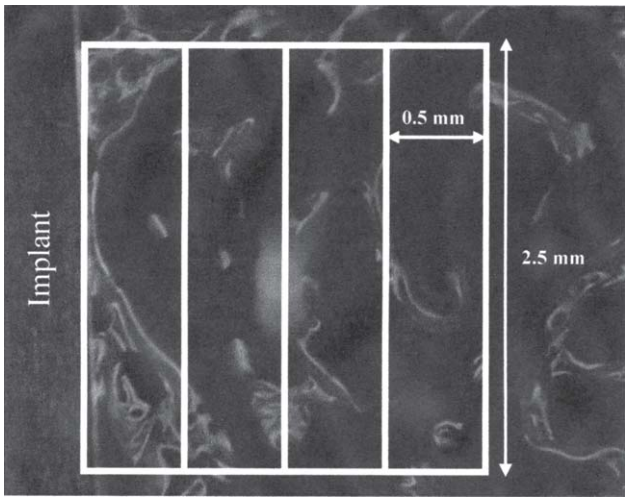

FIGURE 1- Representation of a micrograph (at 40x original magnification) subdivision into 0.5 mm base by 2.5 mm height rectangles for quantification of tetracycline labeled bone area fraction

RESULTS

Surgery and Follow-up

Review and analyses of the surgical procedures and general immediate follow-up demonstrated no significant complications regarding procedural conditions and postoperative infection.

Biocompatibility and Tetracycline Labeling

The merged micrographs revealing the perimeter and bone structure around a 4-week control implant are shown in Figure 2. Qualitative evaluation revealed the presence of bone contact to the implant surface at cortical and trabecular regions for all specimens, and no evidence (at this magnification) of the thin-film bioceramic coating on the surface of experimental groups' implants was found.

Tetracycline labels were found for both control and thin-film coated implants at both times *in-vivo*, and were present at regions in proximity and away from the implant surface.

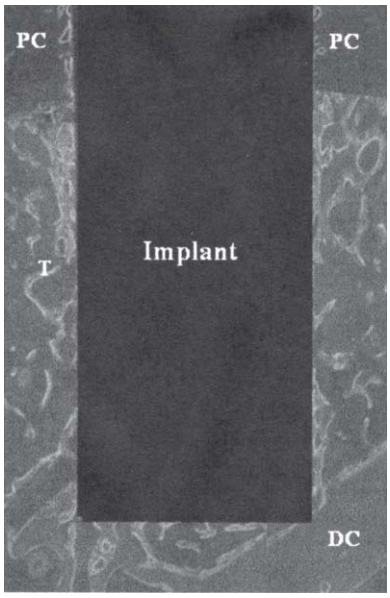

FIGURE 2- Merged micrographs (20x original magnification) revealing perimeter and bone structure around a 4-week titanium alloy (control) implant. Note the presence of tetracycline labels in both cortical and trabecular bone in proximity and away from the implant surface. PC- tibiae proximal cortical plate, DC- tibia distal cortical plate, T- trabecular bone region

Overall Labeling Quantification

Summary statistics for overall labeling quantification are presented in Table 1. These results showed that the thin-film coated implants presented significantly higher values of tetracycline labeled bone area fraction compared to control groups at both times *in-vivo*. Also, the 4-week thin-film coated group presented significantly higher values of tetracycline labeled bone area fraction compared to all other groups. It should be noted that no significant difference was found between the 2- and 4-week control groups.

Labeling Quantification at Region Adjacent to Implant Surface (to 0.5 mm from implant surface)

Tetracycline labeled bone area fraction determination at the region adjacent to the implant surface (to 0.5 mm from implant surface) summary statistics are presented in Table 2. The thin-film coated implants presented significantly higher values of tetracycline labeled bone area fraction at this particular site compared to control groups at both times in vivo. Again, the 4-week thin-film coated group presented significantly higher values of tetracycline labeled bone area fraction compared to all other groups. No significant difference was found between the 2- and 4-week control groups.

DISCUSSION

The results of this study showed that the surgical aspects of this experiment had no significant negative influences due to inflammation and/or infection. Specimen loss had no significant influence in the comparative analyses[12] and was primarily caused by difficulties in nondecalcified specimen preparation.

Evaluation of the merged micrographs rebuilding the implant perimeter and surrounding bone architecture showed that both control and thin-film coated implants are

TABLE 1- Summary statistics for overall tetracycline bone labeled area fraction quantification for thin-film coated (experimental) and titanium alloy (control) at 2 and 4 weeks in-vivo

Group	# of implants	n	Mean % A.F. Labeled	95% C.I.	Std. Deviation	Std. Error	Coeff. of Variance
Control 2 Weeks	7	672	13.56[c]	±1.06	16.72	0.006	0.048
Control 4 Weeks	7	672	14.22[c]	±1.07	16.88	0.007	0.046
Experimental 2 Weeks	7	672	24.04[b]	±1.44	22.62	0.009	0.036
Experimental 4 Weeks	8	768	27.39[a]	±1.45	24.40	0.009	0.032

a,b, and c- statistical group CI overlap at 95% level of significance.

biocompatible and have osseoconductive properties, as *per* direct bone-to-implant contact at regions of cortical (proximal and distal plates) and trabecular bone[1-3]. This phenomenon regarded as osseointegration[2] is a desirable feature when considering endosseous dental implants for functional load bearing applications[1-3,5].

The absence of a thin-film bioceramic coating on the experimental groups' implants indicated that partial or total coating dissolution occurred as implantation time elapsed *in-vivo*. This feature supports opportunities for direct bone contact to the underlying metallic substrate of thin-film coated implants, avoiding the occurrence of weak links between the bioceramic coating and metallic substrate *in-vivo*, where mechanical failure is likely to occur[19-20,22]. The partial or total coating dissolution that occurred *in-vivo* was possibly related to the low thicknesses thin-film coated implants and/or its microstructural phase composition[15,30].

The presence of fluorescent labels at regions in proximity and away from the implant surface in all specimens demonstrated the effectiveness of oxytetracycline as a tissue marker[4,6,13,16,23-24,26], enabling quantification of relative degrees of bone activity around the implants[10,14,27] at both times *in-vivo*.

The overall tetracycline labeled area fraction quantification revealed significantly higher values for the thin-film coated implants compared to control implants at 2 and 4 weeks *in-vivo*, demonstrating a significant effect[7,21,27] of the surface treatment on bone kinetics, as previously reported by different methodologies[8-9,11,17-18] considering bioceramic coatings obtained through different processes.

The site specific (to 0.5 mm from implant surface) tetracycline labeled bone area fraction quantification showed the same qualitative trends within groups when compared to the overall tetracycline labeled area fraction quantification, but presented significant higher values for all groups at both times *in-vivo* (no CI overlaps between identical groups in Tables 1 and 2). These significantly higher values at the region adjacent to the implant surface indicate that the modeling/remodeling rates have higher values compared to regions away from the implant surface, and these values potentially decrease to physiologic levels as a function of distance from the implant surface[10,14,27].

Alteration in bone kinetics found in both overall and site specific tetracycline labeled area fraction quantifications of bone around thin-film coated implants may be beneficial regarding both potential decreases in osseointegration time[14,20,22,29] (short term stability) and long term implant stability maintenance[14]. It is important to note that no relationship between increased bone activity and increased bone-to-implant contact, higher bone-biomaterial interface mechanical properties, or short- and long-term implant treatment success ratios have been presented in a concise manner to date, and both *in-vitro*, *in-vivo*, biomechanical, and controlled clinical research protocols are desirable for addressing these issues. Protocols involving the full physical and chemical characterization of the thin-film coating used throughout this study would also provide valuable insight on relating bone kinetics to coating characteristics, and may be subject of future research.

CONCLUSIONS

Bilateral proximal regions of dog tibia were utilized to study the effect of a thin-film coated (IBAD processed) on bone activity at times 2 and 4 weeks after implantation, and according to the stereological (quantitative microscopy) results obtained, it can be concluded that the Ca- and P-based bioceramic thin-film (IBAD processed) coated implants were biocompatible, osseoconductive, and presented significantly higher overall and site specific (to 0.5 mm distance from implant surface) tetracycline labeled area fraction values on dog's bone.

Direct bone contact to the metallic substrate was achieved for thin-film coated implants while still benefiting from the surface modification's effect on bone kinetics at both times *in-vivo*, supporting the rationale for thin-film coatings as candidates to overcome limitations inherent to commercially available bioceramic coatings processes like PSHA.

AKNOWLEDGEMENTS

This project was partially funded by Bicon Dental Implants, Inc. Boston, MA. This study was the first of a series of histomorphometric, biomechanical, and physico/chemical characterization investigations concerning IBAD

TABLE 2- Summary statistics for tetracycline labeled bone area fraction quantification at the region adjacent to the implant surface (to 0.5 mm) quantification for thin-film coated (experimental) and titanium alloy (control) at 2 and 4 weeks in-vivo

	n	Mean % A.F. Labeled	95% C.I.	Std. Deviation	Std. Error	Coeff. of Variance
Control 2 Weeks	168	17.79[c]	±2.41	18.96	1.463	0.082
Control 4 Weeks	168	17.37[c]	±2.12	16.66	1.285	0.074
Experimental 2 Weeks	168	27.61[b]	±2.94	23.11	1.783	0.065
Experimental 4 Weeks	192	38.40[a]	±3.12	26.22	1.892	0.049

a,b, and c- statistical group CI overlap at 95% level of significance.

thin-film coatings on dental implants.

REFERENCES

1- Albrektsson T, Brånemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone to implant anchorage in man. Acta Orthop Scand 1981;52(2):155-70.

2- Brånemark P-I, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O, et al. Osseointegrated implants in the treatment of edentulous jaws. Experience from 10-year period. Scand J Plast Reconstr Surg 1977;16:1-132.

3- Brånemark P-I. Osseointegrated titanium fixtures in the treatment of edentulousness. Biomaterials 1983;4:25-28.

4- Bevelander G, Makahara H, Rolle GK. Inhibition of skeletal formation in the chick embryo following administration of tetracycline. Nature 1959;184:728-9.

5- Bobbio A. The first endosseous implant in the history of man. Bull History Dent 1973;20:1-6.

6- Boyne PJ, Herford AS. Distraction osteogenesis of the nasal and antral osseous floor to enhance alveolar height. J Oral Maxillofac Surg. 2004 Sep;62(9 Suppl 2):123-30.

7- Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004;83(7): 529-33.

8- Caulier H, Vercaigne S, Naert I. The effect of Ca-P plasma sprayed coatings on the initial bone healing of oral implants. An experimental study in the goat. Biomed Mater Res 1997;34(1):121-8.

9- Chang YL, Lew D, Park JB, Keller JC. Biomechanical and morphometric analysis of hydroxyapatite-coated implants with varying crystallinity. J Oral Maxillofac Surg 1999;57(9):1096-108.

10- Chen J, Chen K, Garetto LP. Mechanical response to functional and therapeutic loading of a retromolar endosseous implant used for orthodontic anchorage to mesially translate mandibular molars. Implant Dent 1995;4:246-58.

11- Cook SD. The effect of surface macrotexture on the mechanical and histologic characteristics of hydroxyapatite- coated dental implants. J Oral Implantol 1993;19(4):288-94.

12- DeHoff R, Rhines F. Quantitative microscopy. McGraw-Hill: New York; 1968.

13- Frost HM, Villanueva AR, Roth H. Measurement of osteoblastic activity in diaphyseal bone. Stain Technology 1960;35:179-90.

14- Garetto LP, Chen J, Parr JA. Remodeling dynamics of bone supporting rigidly fixed titanium implants: a histomorphometric comparison in four species including humans. Implant Dent 1995;4:235-43.

15- Gross KA, Gross V, Berndt CC. Thermal analysis of amorphous phases in hydroxyapatite coatings. J Am Ceram Soc 1998;81:106-12.

16- Guichelaar MM, Malinchoc M, Sibonga JD, Clarke BL, Hay JE.Bone histomorphometric changes after liver transplantation for chronic cholestatic liver disease. J Bone Miner Res. 2003Dec;18(12):2190-9.

17- Hulshoff JE, Jansen JA. Initial interfacial healing events around calcium phosphate (Ca-P) coated oral implants. Clin Oral Implants Res 1997;8(5) 393-400.

18- Jansen JA, van der Waerden JPCM, Wolke JGC. Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbit. J Biomed Mater Res, 1993;27:603–10.

19- Lacefield WR. Hydroxyapatite coatings. In: Hench LL, Wilson J, editors. An introduction to bioceramics. NJ: World Scientific; 1993. p.223-38.

20- Lacefield WR. Current status of ceramic coatings for dental implants. Implant Dentistry 1998;7(4):315-22.

21- Larsson C, Thomsen P, Lausmaa, Rodahl M, Kasemo B, Ericsson LE. Bone response to surface modified titanium implants: studies on electropolished implants with different oxide thicknesses and morphology. Biomaterials 1993,15(13):1062-74.

22- Lemons JE, Dietsh-Misch F. Biomaterials for dental implants. In: Contemporary Implant Dentistry. Misch CE (ed). Saint Louis (Missouri): Mosby Inc; 1999. p.271-302.

23- Milsch RA, Rall DP, Tobie JE. Bone localization of the tetracyclines. Nat Cancer Inst. 1957;19:87-92.

24- Milsch RA, Rall DP, Tobie JE. Fluorescence of tetracycline antibiotics in bone. J Bone Joint Surg Am 1958;40:897-910.

25- Ong JL, Chan DC. Hydroxyapatite and their use as coatings in dental implants: a review. Crit Rev in Biomel Eng 2000;28(5-6):667-707.

26- Recker RR. Bone histomorphometry: techniques and interpretation. CRC Press Inc: Boca Raton, Fl; 1983.

27- Roberts WE. Bone tissue interface. J Dent Educ 1988;52:804-9.

28- Thomas KA, Cook SD. An evaluation of variables influencing implant fixation by direct bone apposition. J Biomed Mater Res 1985;19:875.

29- Young RA. Biological apatite versus hydroxyapatite at the atomic level. Clin Orthop Rel Res 1975; 113:249-62.

30- Zeng H, Lacefield WR. XPS, EDX, and FTIR analysis of pulsed laser deposited calcium phosphate bioceramic coatings: the effects of various process parameters. Biomaterials 2000; 21(1):23- 30.