Determination of osseoactivity gradients around two implant surfaces

Determinação de gradientes de osseoatividade ao redor de duas superfícies de implantes

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Abstract

Incorporation of bioceramics on the surface of dental implants has been utilized in an attempt to increase biological response of bone to materials. The purpose of this study was to investigate osseoactivity gradients as a function of distance from the implant surface for IBAD thin-film bioceramic coated versus sand-blasted/acid-etched titanium alloy implants at 2- and 4- week implantation times in a dog model. Four implants were placed in each tibia and remained for 2 and 4 weeks in-vivo. 10mg/Kg oxytetracycline was administered for labeling purposes. The limbs were retrieved by sharp dissection, and subsequently nondecalcified processed for fluorescent microscopy. Four micrographs (40x mag. subdivided in rectangles) were acquired along one of the implant sides for tetracycline labeled area fraction quantification as a function of distance, and best-fit lines were obtained through computer software. Tetracycline labeled area fraction quantification (osseoactivity) showed the highest values at the region adjacent to the implant surface for all groups, and these values likely decreased to physiologic numbers after some distance. The 4-week thin-film coated implant presented higher osseoactivity values for all distances. A region of highest activity was present to 0.5mm from the implant surface for all groups but the 4-week thin-film coated, which showed an expanded increase to 1 mm from the implant surface. Best line fits revealed higher negative slopes for thin-film coated implants at both implantation times. Activity gradients around dental implants were found to be within 0.5-1mm from the implant surface and were dependent on surface treatment.

Keywords

Gradient, osseoactivity; dental implant, surface sand-blasted; acid-etching, dental tetracycline; dog

Introduction

The desire for replacing missing dental organs have been expressed by mankind since ancient times, as *per* archaeologic findings revealing carved wood, ivory, and metals attempting to restore jaw's function[2]. Dental implantology was practiced with limited success until a series of research papers was published by a Swedish research group[1,3-5], where the term osseointegration was first introduced and defined as direct bone apposition at the surface of either titanium or titanium alloy implants[3-4]. After the establishment of surgical and restorative procedures for implant dentistry therapy, dental implantology became one of the most successful dental treatment modalities with positive outcomes often reported higher than 90%[4-5].

Although dental implant therapy reliability and predictability have been reported since its early days, opportunities for decreasing treatment time (avoiding the 2 stage surgical technique[3-4]) have been sought by both basic scientists and private practitioners. For

that purpose, significant attention has been devoted to altering (accelerating) bone healing kinetics around dental implants.

The parameter most often modified for such purpose has been the implant surface through various surface engineering processes, since it has been reported that these may significantly enhance the bone response to biomaterials[6-7,9-10,14,17,22]. Provided that Ca and P are the major elemental components of bone [9,14,16,18,23], incorporation of bioceramic coatings in the form of apatites and other Ca and P based phases have been investigated in-vitro[13,16,24] and in-vivo[7,9-10,14-15] regarding their elemental and applied properties.

Incorporation of bioceramics in dental implants can be achieved by a variety of processes, including plasma spraying (PSHA), sol-gel, pulsed laser deposition (PLD), ion beam assisted deposition (IBAD), and hot isostatic pressing[16,18]. Among the processes currently available for bioceramic coated implant production, PSHA has been the one used in large commercial scale. Although potential benefits of PSHA coated implants have been reported[7,10,14,15], overcoming process inherent limitations like variable composition (resulting in variable dissolution), coating thickness (20- 50μm, where full dissolution is unlikely to occur in-vivo), and the presence of a metalloceramic interface between the metallic bulk and bioceramic coating (where failure is likely to occur during placement or function) has been subject of various surface engineering investigations[9,13-14,16,18-20].

Potential substitutes for PSHA processed bioceramic coatings are incorporation of coatings of reduced thicknesses known as thin-films (0.5- 5 μm thick[9,13,16,18-19]). Thin-film coatings may also be produced by several surface engineering processes like IBAD, PLD, molecular epitaxial growth, and sol-gel processes[16].

A desirable characteristic for the in-vivo behavior of thin-film bioceramic coated implants is a highly engineered microstructure, providing *in-vivo* tailored dissolution kinetics, resulting in the exposure of the implant metallic substrate some time after implantation. This controlled dissolution supports opportunities for direct bone apposition to the implant surface, avoiding a weak bioceramic link between bone, bioceramic, and metallic substrate, while still benefiting from bioceramics' osseoconductive properties at early implantation times.

It has been demonstrated[9] in laboratory in-vivo studies that the rationale for thin-film coatings happened at early implantation times. In these studies[9], overall and site specific (to 0.5 mm from the implant surface) osseoactivity levels quantified through tetracycline labeled bone[20] area fraction were higher for titanium alloy IBAD bioceramic coated implants compared to sand-blasted/acid-etched titanium alloy implants at two and four weeks implantation times in a dog model. These results indicated that IBAD coated implants altered bone modeling/remodeling dynamics, especially at regions adjacent to the implant surface. Other studies[8,12,21] showed that remodeling rate and bone activity levels were significantly higher along regions closer to implant surfaces compared to regions away from the interface, and that short- and long-term stability of implants may be related to osseoactivity at regions to 1mm away from the implant surface[21].

The purpose of this study was to investigate osseoactivity gradients around thin-film IBAD bioceramic coated titanium alloy implants versus sand-blasted/acid-etched titanium alloy implants at early implantation times in a 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 10mm in length by 4mm in diameter. The number of devices was 32 and included experimental (bioceramic coated, n=16) and control groups (n=16). The manufacturer provided no detail regarding surface topography and chemistry.

Methods

Surgical Model

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

The surgical site was the proximal tibiae, with four implants placed in each limb. Each dog provided a two- and four-week comparison between experi-

mental and control surfaces *per* four-implant location through sequenced surgical procedures. The left limb was used for the four-week evaluation and the right limb for the two-week evaluations. The surgeries were conducted under full anesthesia and following sterile methodologies.

Surgical Implantation

The proximal tibiae was exposed subperiostally, 4 equi-spaced holes were drilled through sequential burs (external irrigation), and the implants were inserted into the trabecular mid-region with the top of the implant in contact with the cortical surface. A polymeric cover screw was threaded into the implant top and standard layered procedures were employed for soft tissue closure.

48 hours prior to euthanization, a 10mg/kg subcutaneous oxytetracycline was administered to provide fluorescent labeling for histomorphometric analyses (single label).

Specimen Preparation

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

Tetracycline Labeling Quantification as a Function of Distance From Surface

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 were subdivided into rectangles (0.5mm base, 2.5mm height) comprising 0.5mm steps from the implant surface, and a nine-point grid was randomly placed 6 times for each micrograph subdivision for stereological inferences[11]. This procedure implied 24 tetracycline labeled bone area fraction measurements for each micrograph and a total of 96 measurements per implant.

Bone Activity Gradient Determination

Activity gradient assessment was performed by computer software (Microsoft Excel, WA- USA). The best-fit lines (linear model), equations for activity as a function of distance, and R^2 statistical parameters were obtained through plotting area fraction labeled percent as a function of 0.5 mm steps from the implant surface for both groups and times in-vivo.

Statistical Analyses

The confidence interval (CI) for each parameter evaluated 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 statistical level of significance, and n= number of observations for the parameter under evaluation[11].

Results

The summary statistics parameters for tetracycline labeled area fraction as a function of distance from the implant surface for both groups and times in-vivo are presented in Table 1. Note that the highest tetracycline labeled area fraction values for all groups were at regions adjacent to the implant surface, and these values decreased as a function of distance from the implant surface. The 4-week experimental group presented the highest values of tetracycline labeled area fraction for each interval evaluated compared to all other groups.

The best-fit line of tetracycline labeled area fraction as a function of distance (activity gradient) for each group is presented in Figures 1 and 2 for control and experimental groups respectively. The lines obtained for the experimental groups presented higher negative slope values compared to control groups. Line equations, R^2 values, and negative slope values are presented in Table 2 for the different groups evaluated. The R^2 values obtained for control groups presented higher values compared to experimental groups. The x=0 intercept values obtained from the equations on Table 2 were 20.31, 20.32, 28.77, and 41.91 for the 2-week control, 4-week control, 2-week experimental, and 4 week-experimental groups respectively.

Table 1 – Statistics Summary for tetracycline labeled area fraction as a function of distance from the implant surface for control and experimental groups at 2 and 4 weeks in-vivo

Group	Distance From Implant Surface	# of Observations	Mean	95% CI	Std. Dev.	Std. Error
2-Week Control	0.5 mm	168	17.79[a,3]	±2.40	18.96	1.46
	1 mm	168	12.93[b,4]	±2.19	17.28	1.33
	1.5 mm	168	9.83[b,4]	±2.49	19.63	1.51
	2 mm	168	7.88[b,4]	±2.24	17.67	1.36
4-Week Control	0.5 mm	168	17.37[a,3]	±2.11	16.66	1.28
	1 mm	168	13.91[b,4]	±2.02	17.04	1.23
	1.5 mm	168	9.93[b,4]	±2.07	16.33	1.26
	2 mm	168	8.01[b,4]	±2.34	18.4	1.42
2-Week Experimental	0.5 mm	168	27.61[a,2]	±2.93	23.11	1.78
	1 mm	168	12.45[b,4]	±2.40	18.92	1.46
	1.5 mm	168	11.38[b,4]	±2.12	16.72	1.29
	2 mm	168	10.13[b,4]	±2.42	19.05	1.47
4-Week Experimental	0.5 mm	192	38.4[a,1]	±3.11	26.22	1.89
	1 mm	192	19.09[b,3]	±2.24	18.84	1.36
	1.5 mm	192	13.36[c,4]	±2.54	21.33	1.54
	2 mm	192	12.07[c,4]	±1.93	16.21	1.17

a,b,c - within each group; 1,2,3- among all groups.

Table 2 – Best-fit line equation and R^2 values for control and experimental groups at 2 and 4 weeks in-vivo. Note the negative slope values deriving from best fit line equations

Group	Best Line Fit Equation	R^2	Negative Slope
2-Week Control	y = -3.283x + 20.31	0.98	3.28
4-Week Control	y = -3.206x + 20.32	0.96	3.21
2-Week Experimental	y = -5.351x + 28.77	0.71	5.35
4-Week Experimental	y = -8.472x + 41.91	0.81	8.47

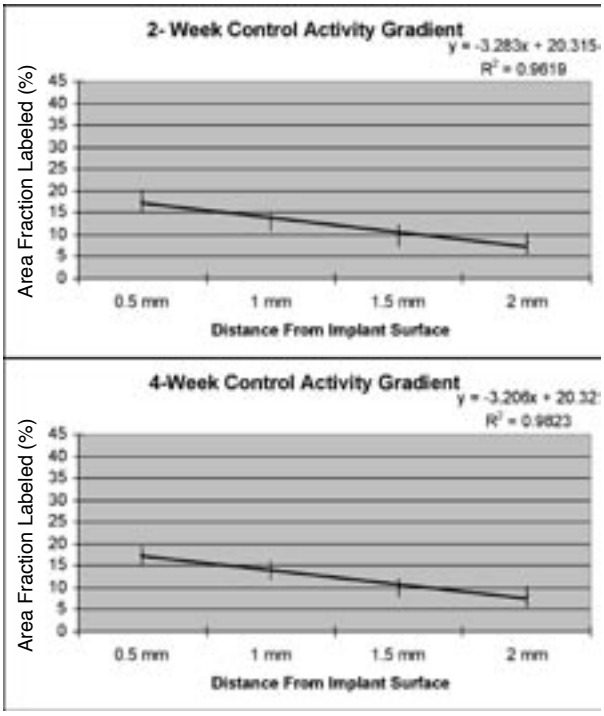

FIGURE 1 – Best-fit line for Area Fraction Labeled (%) as a function of distance from the implant surface for sand-blasted/acid-etched (control) groups.

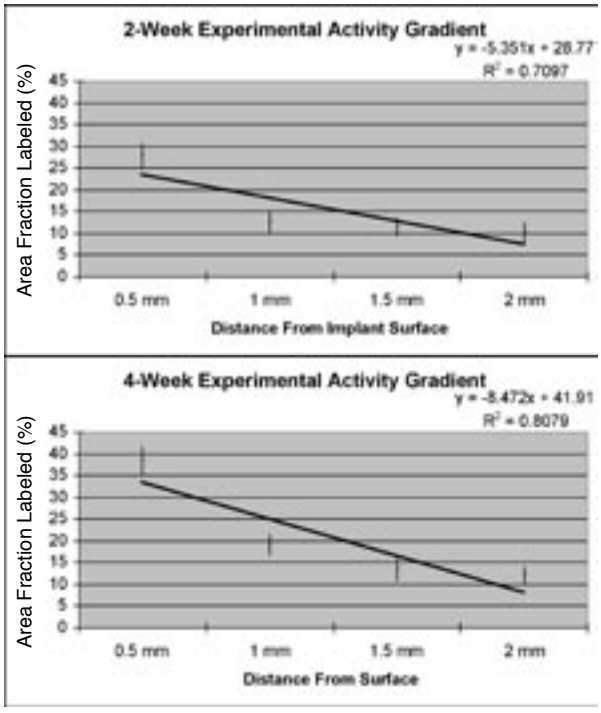

FIGURE 2 – Best-fit line for Area Fraction Labeled (%) as a function of distance from the implant surface for thin-film coated implant (experimental) groups.

Discussion

Biocompatible and osseoconductive[1-5] properties have been previously reported for both thin-film IBAD bioceramic coated implants[9] (experimental group) and sand-blasted/acid-etched (control) implants[6,9]. Specimen losses had no significant influence on the comparative analyses[11] and were caused due to difficulties in specimen preparation.

The results presented in Table 1 showed the presence of a region of highest activity (site specific) to 0.5mm from the implants' surface for all implant groups. This region has been shown to have higher bone activity from short to long-term implantation times [8,9,12,21] compared to regions away from the implant surface, and has been regarded as important for implant short- and long-term maintenance[21]. The experimental groups presented significantly higher tetracycline labeled[20] area fraction at regions adjacent to the implant surface (to 0.5mm from surface) compared to control groups at both times in-vivo, revealing coating dissolution effects on bone activity levels. Osseoactivity values decreased as a function of distance for all groups, and indication that these values likely reached physiologic numbers[9] after 0.5mm away from the implant surface was provided by CI overlaps after this distance for the 2-week control, 4-week control, and 2-week experimental groups.

The 4-week experimental group was the only group to present significantly higher tetracycline labeled area fraction at the region from 0.5 to 1 mm from the implant surface compared to other groups. This significantly higher value revealed a time-dependent expansion of the thin-film bioceramic coating effect[6-7,9-10,14,17,22] (attributed to coating dissolution in-vivo[9,13,16,18-19,23]) on bone, and may possibly accelerate bone modeling/remodeling while in function, supporting opportunities for early loading of thin-film coated implants. The osseoactivity levels for the 4-week experimental group overlapped with other groups at regions further than 1 mm away from the implant surface, further suggesting decreases in osseoactivity to physiologic levels. The results obtained in this study were in qualitative agreement with another study[9] where labeling quantification was performed by a different methodology for the same implantation times *in-vivo*.

The assessment of bone activity gradients around implants through best-fit line equations showed higher negative slopes for the experimental groups compared to control groups at both times *in-vivo*, providing more evidence of thin-film coating effects on osseoactivity. The relative magnitudes of the negative slopes increased as a function of time in-vivo and was due to the highest value of tetracycline labeled area fraction in this study, obtained for the interval region adjacent to the 4-week experimental group. It must be noted that line fits were more accurate for control groups compared to experimental groups at R^2 values of 0.98, 0.96, 0.71, and 0.81 for the 2-week control, 4-week control, 2-week experimental, and 4-week experimental groups respectively. The higher R^2 values for control groups are due to the lower tetracycline labeled area fraction values at the region adjacent (to 0.5mm) to the implant surface, which were not much higher than values obtained for regions further than 0.5mm from the implant surface for these groups, providing smoother line fits during analyses. The line fit for the experimental groups were poorer due to the significantly higher values of tetracycline labeled area fraction presented at regions adjacent to the implant surface at both times in-vivo, which caused steeper steps from 0.5mm to 1mm, and from 1 mm to 1.5mm for the 2-week and 4-week experimental groups respectively. The x=0 values obtained from the equations presented in Table 2 may only be regarded as a qualitative extrapolation of osseoactivity levels at the bone-biomaterial interface, once discrete intervals were taken for activity gradients investigation.

Analyzing the discrete data presented in Table 1 along with best-fit lines obtained for the different groups at both times in-vivo, osseoactivity gradients were found to exist, but not to extend to 2mm (until reaching physiologic levels) from the implant surface. According to these results, gradients were possibly confined to regions to 0.5mm away from the implant surface, once decreases in values potentially achieving physiologic numbers occurred after this length into the bone for the 2-week control, 4-week control, and 2-week experimental groups. It should be noted that even within this narrow interval, activity gradients for the experimental groups would be steeper compared to control groups at both times in-vivo. The only instance where an expansion of the gradient length occurred was for the 4-week experimental analysis, and this expansion was due to a time-dependent increase in osseoactivity provided by the thin-film bioceramic coating at the region from 0.5 to 1mm away from the implant surface.

Conclusions

According to the results obtained in this investigation, activity gradients on bone around dental implants were found to be within 0.5 to 1mm away from the implant surface before reaching physiologic activity levels. Activity gradients were steeper for thin-film coated implants compared to grit-blasted/ acid etched implants due to the significantly higher activity levels provided by bioceramic coating influence at regions adjacent to the implant surface. Also, a time-dependent influence due to bioceramic effects on bone kinetics extended the gradient to 1mm from the implant surface for the 4-week thin-film coated implants.

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Resumo

A incorporação de biocerâmicas na superfície de implantes dentários tem sido utilizada na tentativa de se aumentar a resposta biológica aos materiais. O propósito desta investigação foi analisar gradientes de osseoatividade em função da distância da superfície de um implante com recobrimento de surperfície de baixa espessura depositado com auxílio de feixe iônico comparado a um implante com jateamento de óxido de alumínio/ banho ácido em 2 e 4 semanas de implantação em um modelo animal laboratorial. Quatro implantes foram colocados em cada tíbia, onde permaneceram por 2 e 4 semanas in-vivo. 10 mg/Kg de oxitetraciclina foram administradas como marcador biológico. As tíbias foram dissecadas e subsequentemente processadas para análise de fluorescência em microscópio. Quatro microfotografias (magnificação de 40 vezes, divididas em retângulos) foram tiradas ao redor de um dos lados do implante para quantificação da área marcada por tetraciclina em função da distância da superfície do implante, e equações lineares foram obtidas com a ajuda de um programa de computador. A quantificação da área marcada por tetraciclina (atividade óssea) mostrou valores maiores na região adjacente à superfície do implante para todos os grupos, e esses valores diminuiram com o aumento da distância. Os implantes com recobrimento de baixa espessura (grupo de 4 semanas) apresentaram maior atividade óssea em todas as distâncias. O intervalo até 0.5mm da superfície do implante foi a área de maior atividade óssea em todos os grupos, exceto no grupo de 4 semanas com recobrimento de baixa espessura, onde a área de maior atividade foi expandida até 1mm da superfície do implante. Equações lineares revelaram inclinações negativas de maior magnitude para recobrimento de baixa espessura durante os dois períodos avaliados. O gradiente de atividade ao redor dos implantes foi entre 0.5 e 1mm da superfície do implante e mostrou-se afetado pelo tratamento de superfície.

Palavras chave

Gradiente, atividade óssea; implante dentário, superfície; ataque-ácido dentário; tetraciclina; cão.

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