*Molecular Assessment of Endosseous Implant Adherent Cellular Phenotypes in Man*

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## ABSTRACT

Matthew S. Bryington, D.M.D.: Molecular Assessment of Endosseous Implant Adherent Cellular Phenotypes in Man
(Under the direction of Lyndon F. Cooper, D.D.S., Ph.D.)

This investigation considered the role of implant surface roughness on activation of osteoblastic differentiation of uncommitted mesenchymal stem cells in humans. The aim of this study was to examine the gene expression profiles from adherent cells on moderately roughened $TiO_2$ grit-blasted (micron-scale topography) and hydrofluoric acid modified $TiO_2$ grit-blasted (nano-scale topography) implants. Sixty cpTitanium implants were placed and allowed to integrate for 24 hours, 72 hours, and one week in 10 systemically healthy patients. These implants were retrieved and biochemical analyzed by RT-PCR for markers of osteoblastic differentiation. A complex array of gene changes representative of osteogenesis was observed at both implant surfaces. Compared to implants with micron-scale topography, nano-scale topography implants supported greater expression of RUNX-2(1.6 vs. 2.6-fold, p=0.18), Osterix (1.8 vs. 3.9-fold, p=0.04), BSP(2.7 vs. 4.6-fold, p=0.11) and OCN(1.6 vs. 3.2-fold, p=0.06) mRNAs indicating greater osteogenesis at 7 days *in vivo*. Further investigations using this model may elucidate important difference among subsets of patients who experience implant failure.

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# CHAPTER 1

## INTRODUCTION

Endosseous implants are of widespread importance in the medical and dental profession. It has been widely demonstrated that implant surface plays a major role in mediating the bone-to-implant interface (Albrektsson and Johansson 2001). The importance of osseointegration in clinical dentistry has been accepted as the basis of implant success (Albrektsson 2001). The high implant success rates achieved in the dense bone present in the parasymphaseal mandible and have not been achieved in other more clinically difficult situations (Shalabi, Gortemaker et al. 2006). Also, for a subset of patients such as smokers (Bain 1996) and diabetics (Fiorellini, Chen et al. 2000), there are anecdotal reports of difficulty achieving high implant success rates. These failures, while not specifically determined, have been attributed to failure of bone formation leading to osseointegration. Several factors have been associated as being involved with failure of osseointegration including, anatomic, local biologic, and systemic factors (Shalabi, Gortemaker et al. 2006; Mendonca, Mendonca et al. 2008). Control of these complex factors has been a central focus of implant research for several decades (Mendonca, Mendonca et al. 2008). These findings have lead to a large investment in dental implant surface technologies as a means to promote and achieve greater osseointegration (Mendonca, Mendonca et al. 2008).

In 1990 AstraTech Dental released the TiOblast$^{TM}$ implant. The TiOblast$^{TM}$ surface was a commercially pure titanium implant that has been sprayed with titanium

dioxide ($TiO_2$) (Hansson and Norton 1997). This produced a moderately roughened grit blasted surface with micron level surface topography(Hansson and Norton 1999). The importance of micron topographic surfaces was demonstrated in a study by Buser and colleges where he compared a moderately roughened surface to a polished machined surface and a hydroxyapatite rough surface (Buser, Schenk et al. 1991). He observed that the moderately roughened surface was capable of faster and greater bone accrual as compared to the positive and negative controls. This has lead to the use of micron topographic implants in the clinical setting and has provided the TiOblast™ implant with one of the longest clinical follow-up periods in the dental literature (Albrektsson and Wennerberg 2004).

The TiOblast™ surface was the precursor to the AstraTech Dental Osseospeed™ (released 2004) surface which was a further advancement of the TiOblast™ moderately roughened surface. It was produced by chemically treating the $TiO_2$ grit blasted TiOblast™ implant with hydrofluoric acid (HF) which results in nanometer-sized topographical features as well as incorporating fluoride ions onto the $TiO_2$ surface. The use of nanotopographic surfaces demonstrates an entrance into nanotechnology which was defined by the National Aeronautics and Space Administration as, "the creation of functional materials, devices and systems through control of matter on the nanometer length scale (1-100 nm), and exploitation of novel phenomena and properties (physical, chemical, and biological) at that length scale."(Mendonca, Mendonca et al. 2008) The application of nanotopographic surface features on dental implants typically involves a two dimensional association of the features occurring both across and away from the mean surface of the implant. These features can be developed in two arrangements

either isotropic (organized manner) or anisotropic (unorganized manner). Due to the technical complexities inherent in implant design most nanofeatures in implant dentistry are anisotropic (Mendonca, Mendonca et al. 2008).

Several *in vitro* and *in vivo* studies have implicated that nanotopographic implant features can enhance (1) the physical interaction of the implant with bone, (2) the rate of bone formation at the implant surface, and (3) increases the implant survival rate in various situations (Mendonca, Mendonca et al. 2008). It is believed that these effects can be attributed to complex changes not just at or on the implant surface topography but the surface chemistry as well. It is often considered a complication of nanoscale manipulation that the surface will acquire unique chemical properties that are not inherent in the bulk material (Mendonca, Mendonca et al. 2008). Some theorize that imparting nanotopography to dental implants may allow for biomimicry of the cellular environment (Palin, Lui et al. 2005). An example of this being the epithelial basement membrane which contain pores that are approximately 70-100 nm (Brody, Anilkumar et al. 2006); similarly it has been suggested that surface bone roughness is approximately 32 nm. These examples lend credence that cellular structures may contain nanotopographic elements.

Cells are able to detect and react to nanostructures. It has been found that cells react to these nanotopographic features by both a direct and indirect manner (Mendonca, Mendonca et al. 2008). Indirect interactions with cells are mediated by increased surface protein adherence (Mendonca, Mendonca et al. 2008). Direct interactions are mediated by integrin receptors which interact with the implant surface to control adhesion, spreading, and motility (Berry, Dalby et al. 2006; Mendonca, Mendonca et al. 2008).

The molecular phenotype of cells adherent to endosseous implants can be measured after explantation. Early retrieval of endosseous dental implants has permitted the morphologic, biochemical and molecular assessment of the adherent cellular phenotype (Sul, Johansson et al. 2005). The advent of real-time Polymerase Chain Reaction (RT-PCR) allows for the measurement of gene expression at the mRNA level by permitting assessments from only a few cells and enables a comprehensive evaluation of osteoinductive and bone-specific gene expression events in cells adherent to explanted endosseous dental implants (Guo, Padilla et al. 2007).

Early explanation of experimental implants from humans is possible. A human model of osseointegration using healthy volunteers has provided access to histological data following up to 6 months of healing followed by explantation and histological assessment (Ivanoff, Widmark et al. 2003). This model involved trephination of the implant and surrounding bone. Rocci and Colleagues also performed a histological comparative study examining bone formation around TiOblast (micron topography) implants and Osseospeed (nanotopography) implants (Rocci, Rocci et al. 2008). At eight and twelve weeks they found that bone accrued faster and with greater volume around nanotopography implants in humans.

In the current project, the molecular phenotype of cells adherent to endosseous implants retrieved from human volunteers were evaluated by a) isolation of mRNA from the adherent cells, b) synthesis of cDNA from the isolated mRNA by reverse transcription, and c) measurement of the steady state level of targeted, relevant osteoinductive and bone-specific mRNAs by RT-PCR.

Adherent cell molecular phenotypes can be quantitatively compared among surfaces by RT-PCR(Livak and Schmittgen 2001) (Guo, Padilla et al. 2007). The molecular assessments performed by RT-PCR can be highly quantitative. The data is also normalized to a set of so-called housekeeping genes that are thought to be expressed at relatively constant levels throughout the diverse physiological changes of the cell's lifetime. Thus, expression of data by statistical methods permits a kinetic and comparative analysis of gene expression among the two test surfaces.

This study involved explantation of endosseous dental implants following one to seven days of healing by reverse threading of the implants. It is possible to evaluate molecular changes in the phenotype of adherent cells that occur during the important first days of the osseointegration process. Experimental implants were surgically placed in healthy volunteers and explanted after one, three, and seven days for quantitative, kinetic-analysis of gene expression of seven different osteoinductive and bone-specific genes in the implant adherent cells. This will provide the first molecular assessment of the early wound healing and osseointegration process in man and may provide important new molecular information regarding improvements in dental implant surface characteristics to promote or support the process of osteoinduction.

# CHAPTER 2

## MATERIALS AND METHODS

A total of 10 systemically healthy individuals were enrolled in an approved clinical study by the Institutional Committee on Human Subjects Research at the University Of North Carolina School Of Dentistry (IRB Protocol #08-0941). Subjects were required to be between the ages of 18 and 70, be able to provide informed consent, and had sufficient interradicular space to allow for implant placement without damaging sensitive anatomical structures. Subjects were excluded if they presented with a major systemic disease (HTN, diabetes, CVD, etc.), pregnant, or taking medications that affected bone healing. Candidate subjects that met the selection criteria of the study were provided with a clinical examination and panoramic radiologic analysis. A signed informed consent approved by the institutional review board was obtained for each patient.

Implant sites were selected to provide implant placement of the implants into interradicular sites that would not impinge on teeth, nerves, or blood vessels. Six experimentally designed implants were placed into three prepared surgical sites with each site receiving a microtopography and nanotopography implant. Implants were fabricated by AstraTech Dental (AstraTech, MoIndal, Sweden) from Type IV commercially pure titanium and were 2.2 mm wide by 5.0 mm long (**Fig 1**). Implants were surface treated to

impart either a $TiO_2$ grit blasted micro roughened surface (AstraTech, TiOblast proprietary surface) used as a control or a hydrofluoric acid $TiO_2$ grit blasted nanotopographic experimental surface (AstraTech, OsseoSpeed proprietary surface). Surface treatment of implants was verified by SEM analysis performed internally by AstraTech. **Figure 2** shows an SEM analysis of an OsseoSpeed 2.2 x 5.0 mm implant.

All subjects were given presurgical medications of 1 g amoxicillin, 800 mg ibuprofen, and chlorohexidine rinse for 30 seconds. Adequate surgical sites were selected and three full thickness incisions were made (one incision per experimental site), tissues were elevated by a mucoperiosteal elevator under local anesthesia with lidocaine 2% with epinephrine (1:100,000) or mepivacaine 4%. Two osteotomies were prepared per surgical site using a single 2.0mm twist osteotomy drill, prepared to 5 mm depth utilizing sterile saline irrigation (**Fig 3**). The control implants (TiOblast) were placed in the most superior osteotomy site by a self-tapping procedure using hand torque wrenches. Experimental implants (HF modified $TiO_2$ grit blasted implants) were placed in the most inferior osteotomy site. Bone quality and quantity assessments were made at time of surgery to insure primary stability at time of placement (**Fig 4**). Each site was sutured to allow primary closure with 4.O chromic gut suture. For each time point: 24 hours, 72 hours, and one week, one of the three surgical sites was re-exposed and the implants were removed by reverse threading utilizing an Astra prosthetic driver (**Fig 5**). Explantation site selection was based on clinical signs of tissue healing to allow for the least trauma to gingival tissues.

Explanted implants were immediately rinsed in neutral phosphate buffered saline (PBS) to remove blood products and non-adherent cells. Implants were then placed in Trizol Reagent (Invitrogen, Carlsbad, CA), cell lysates were snap frozen in dry ice, and stored at -80$^{o}$C until further use. Total RNA was isolated utilizing the Trizol protocol and precipitated with ethanol according to manufacturer's instructions. Total RNA was quantified by Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE).

For each total RNA sample, cDNA was generated utilizing Superscript$^{TM}$ III reverse transcriptase (Invitrogen, Carlsbad, CA) in a 20 μL reaction. Equal volumes of cDNA were used to program a RT-PCR array (SA Biosciences, Frederick, MD) encoded for mRNAs of Osterix, RUNX-2, BSP, BMP6, ALP, OCN, OPN, and GAPDH. Reactions were performed using a TaqMan Universal PCR Master Mix with an ABI 7000 RT thermocycler for SYBR Green absorbance. Normalizing to GAPDH abundance, relative mRNA abundance was determined by the $2^{-\Delta\Delta Ct}$ method and reported as fold induction (Livak and Schmittgen 2001; Guo, Padilla et al. 2007).

## STATISTICAL ANALYSIS

Descriptive statistics were calculated utilizing Microsoft Excel. SABiosciences $RT^2$ Profiler$^{TM}$ PCR Array Data Analysis software was utilized to analyze RT-PCR data, and generate results by the $2^{-\Delta\Delta Ct\ method}$. Tukey's test was used to evaluate the difference between mRNA levels on each implant surface per time point to the 95% confidence level ($p<0.05$).

# CHAPTER 3

## RESULTS

Markers of osteoblastic differentiation were detected to be expressed in all implant samples at each time interval. Following implantation, a complex interaction of molecular interactions occurred at the implant surface.

Fold induction, the ratio of experimental activity compared to the control activity, was calculated for all samples by normalization to GAPDH expression. **Graph 1** illustrates measurement of mRNA levels at day seven. All samples were found to have greater gene expression around the nanotopography HF modified $TiO_2$ grit blasted surface (Osseospeed). To determine the effects of nanotopographic surfaces on osteogenesis the osteogenic transcription factors Osterix and RUNX-2 were evaluated (**Graph 2**). At seven days, the mRNA levels of Osterix demonstrated a significant increase ($p=0.04$) on nanotopographic surfaces (3.94-fold) as compared to the microtopographic surfaces (TiOblast) (1.84-fold). RUNX-2 demonstrated a similar preference for nanotopographic surfaces (micron 1.61 vs. nano 2.65-fold) but failed to reach significance ($p=0.18$). These findings are in agreement with previous *in vitro* and *in vivo* animal studies that demonstrated greater mRNA levels around nanotopographic implants (Mendonca, Mendonca et al. ; Guo, Padilla et al. 2007; Mendonca, Mendonca et al. 2009; Mendonca, Mendonca et al. 2009).

For several genes the gene expression on microtopographic surfaces exceeded the corresponding expression at 1 week at the 72 hour time point. As can be seen on **Graph 3** and **Graph 4**, Osteocalcin (OCN), BMP6, BSP, and Osterix (OSX) all followed a pattern of greater expression of mRNAs on TiO2 grit blasted surfaces at 72 hours as compared to gene expression at 1 week. Interestingly, gene expression on microroughend surfaces actually exceeded expression on nanotopographic surfaces for OCN, BMP6, and OSX. However, statistical analysis revealed that none of these differences achieve statistical significance.

# CHAPTER 4

## DISCUSSION

The examination of wound healing around dental implants has been of scientific interest for several decades. Numerous *in vitro* and histological studies have suggested that implant surface characteristics play an essential role in mitigating this interaction (Albrektsson and Wennerberg 2004; Albrektsson and Wennerberg 2004). This study represents the first *in vivo* molecular biological analysis in humans demonstrating that implant surface does have a role in affecting the gene expression profiles of adherent cells on cpTitanium implants.

In the present study ten systemically healthy patients were enrolled from the patient population at the University of North Carolina at Chapel Hill. These patients received six surgically placed 2.2 X 5mm experimental micro implants that were imparted with either a $TiO_2$ grit blasted microtopographic surface or a HF modified $TiO_2$ grit blasted nanotopographic surface. Both of these surfaces correspond to the proprietary AstraTech Dental (MoIndal, AB) TiOblast and Osseospeed surfaces respectively. Implants were removed by reverse threading at one day, three days, and one week post implantation.

In this study all markers of osteoblastic gene expression showed increased expression on nanotopgraphic surfaces at 1 week as compared to micron topographic surfaces. Previous *in vitro* studies have shown that osteoblastic gene expression on $TiO_2$

and $TiO_2$/HF surfaces occurs sooner than on polished machine surface (Guo, Padilla et al. 2007). This study supports these findings by demonstrating osteoblastic gene expression at 3 days post insertion.

As previously stated, mRNA levels for Osteocalcin (OCN), BMP6, BSP, and Osterix (OSX) were greater on microroughened surfaces at 72 hours as compared to 1 week. There are several possibilities that could cause this pattern of mRNA expression including a response to pro-inflammatory markers, statistical error as result of small sample size, or an otherwise unknown biological process. Additional research would be required to elucidate a possible mechanism for this phenomenon.

The examination of osteoblastic transcription factors has been utilized in several studies to elucidate the role implant surfaces may play on osteogenesis. In this study we examined the key osteogenic transcription factors of RUNX2 and Osterix. Previous knockout mice studies have demonstrated that both of these transcription factors are required for the development of bone (Nakashima, Zhou et al. 2002). RUNX2 functions as a major osteoblastic transcription factor considered necessary to initiate differentiation of undifferentiated human mesenchymal stem cells into osteoblast precursor cells (Komori). Osterix functions downstream of RUNX2 and is considered the master osteogenic transcription factor, it is considered to be the essential factor determining if a cell will become an osteoblast or a chondroblast (Nakashima, Zhou et al. 2002). In the present study, both RUNX2 (1.6- vs. 2.6-fold, p=0.0) and Osterix (1.8 vs. 3.9-fold, p=0.04) were found to be expressed at greater levels on HF modified nanotopgraphic surfaces. The observation that Osterix is expressed at significantly greater (p=0.04) levels on nanotopgraphic surfaces lends itself to the concept that HF modified $TiO_2$ grit

blasted nanotopographic surfaces are better capable of supporting osteoinduction of adherent cells.

This study lends support to previous studies that examined TiO2/HF surfaces effects on MC3T3-E1 cells. The MC3T3-E1 cell is a murine calvarial osteoprogenitor cell line that is considered to be a committed osteoprogenitor. It is routinely utilized to study osteogenesis through several important ostrogenic signaling pathways (ex., BMP, RUNX-2, PTH, Wnt and Dlx-5). That study found that TiO2/HF nanotopographic implants demonstrated greater expression of both the RUNX-2 and Osterix transcription factors at 3 and 7 days suggesting that the HF acid modified surface could better support osteoinduction. (Guo, Padilla et al. 2007)

When compared to cultured osteoblastic cell studies, the induction of osteogenic markers on nanotopgraphic implant surfaces in humans may further indicate a specific influence on early populations of undifferentiated cells. The presence of greater mRNA levels of Osterix can lead to the conclusion that greater osteoinduction occurs in cells adherent to nanotopographic implants. This helps to explain earlier histological analysis where bone formation around nanotopographic implants was demonstrated to occur much quicker as compared to $TiO_2$ microtopographic implants (Rocci, Rocci et al. 2008).

A limitation in this study is that the variation in gene expression among individuals is not known. While it is possible to derive statistically meaningful data from as few as 4 or 5 inbred strains of rodents, it may require as many as 10 – 12 human volunteers for robust statistical analysis (Boyle, Rosengren et al. 2003). Another

limitation is that we are only analyzing cells adhered to the implant surface which may represent only a fraction of cells participating in wound healing and osseointegration.

# CHAPTER 5

## CONCLUSIONS

In conclusion this study represents the first biomolecular analysis of early implant healing in humans. Previous studies have only focused on histological analysis. This study also demonstrates that it is possible to analyze molecular interactions occurring around dental implants in human subjects and that nanoscale modification of titanium surfaces do in fact alter behavior of adherent cells. Indeed, Osterix transcription factor was significantly expressed at higher levels at 1 week with nanotopographic implants than microtopographic implants. This could be indicative of greater osteoblastic differentiation around implants which explains the more rapid bone formation observed by histology. This study also indicates that enough total RNA can be obtained for future research interests such as gene array analysis of adherent implant cells and molecular analysis of implant failure in specific subsets of patients that have greater incidence of failure.

This study shows that implant surface topography does play a role in mediating the function of osteoblasts which could indicate greater or faster osseointegration. By demonstrating that nanotopographic surface modifications do interact with peri-implant cells, it helps to validate the need for implant surface research and could lead to surface technologies that promote even greater osseointegration. Utilizing this method it would also be possible to evaluate other commercially available implant surfaces and determine if the observed surface interactions are exclusive to a hydrofluoric acid modified

nanotopographic surface or if other implant surfaces can mediate these interactions. This technique also lends itself to possibly determining the molecular basis of implant failure by examining cell to implant interactions in subsets of patients that routinely have poor implant success (e.g. smokers and diabetics). By examining this subset in comparison to healthy individuals it may elucidate the cause for implant failure and allow for the design of implants that can overcome this.

# CHAPTER 6

TABLES AND FIGURES


Figure 1

The AstraTech Dental 2.2 x 5.0 mm experimental implant. Six of these implants were placed in each of ten systemically healthy individuals. This implants surfaces were either treated by TiO2 grit blasting to impart a micron scale topography surface or TiO2 grit blasting followed by modification with hydrofluoric acid to impart a nano scale topography surface.

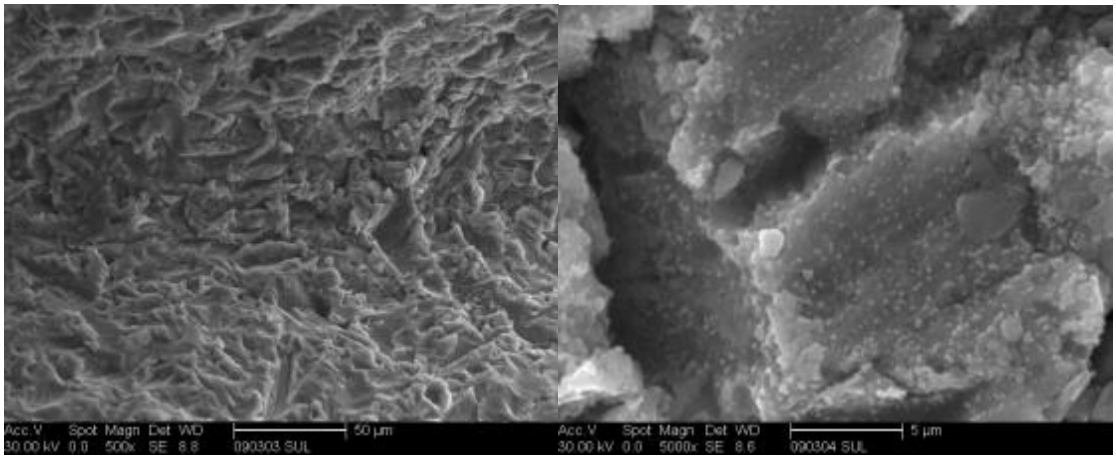



**Figure 2**

SEM Photograph of experimental 2.2 x 5.0mm HF modified TiO2 grit blasted implant at 500x (left) and 5000x (right) magnification. Note the inclusion of pits and grooves present on the surface of the implant at the 5000x magnification. The anisotropic nature of the nanotopographic surface is easily discernible

| | TiOblast 72 hr | Osseospeed 72 hr | TiOblast 1 wk | Osseospeed 1 wk |
|---|---|---|---|---|
| ALP | -1.0371 | -1.3172 | -1.9003 | -1.2027 |
| OCN | 2.5937 | 2.5535 | 1.576 | 3.1629 |
| BMP6 | 2.256 | 1.858 | 1.1388 | 1.8198 |
| BSP | 4.1411 | 4.1518 | 2.6735 | 4.6468 |
| RUNX2 | 1.8564 | 2.4773 | 1.6105 | 2.6482 |
| OPN | 4.9717 | 3.0684 | 7.1046 | 7.9931 |
| OSX | 4.7899 | 3.4313 | 1.8404 | 3.9449 |
| GAPDH | 1 | 1 | 1 | 1 |

Table 1

The table of fold induction for all genes at 72 hours and 1 week post implantation. Fold induction is calculated by using TiOblast 24hour samples as a control. GAPDH is utilized as a control gene to allow for standardization.

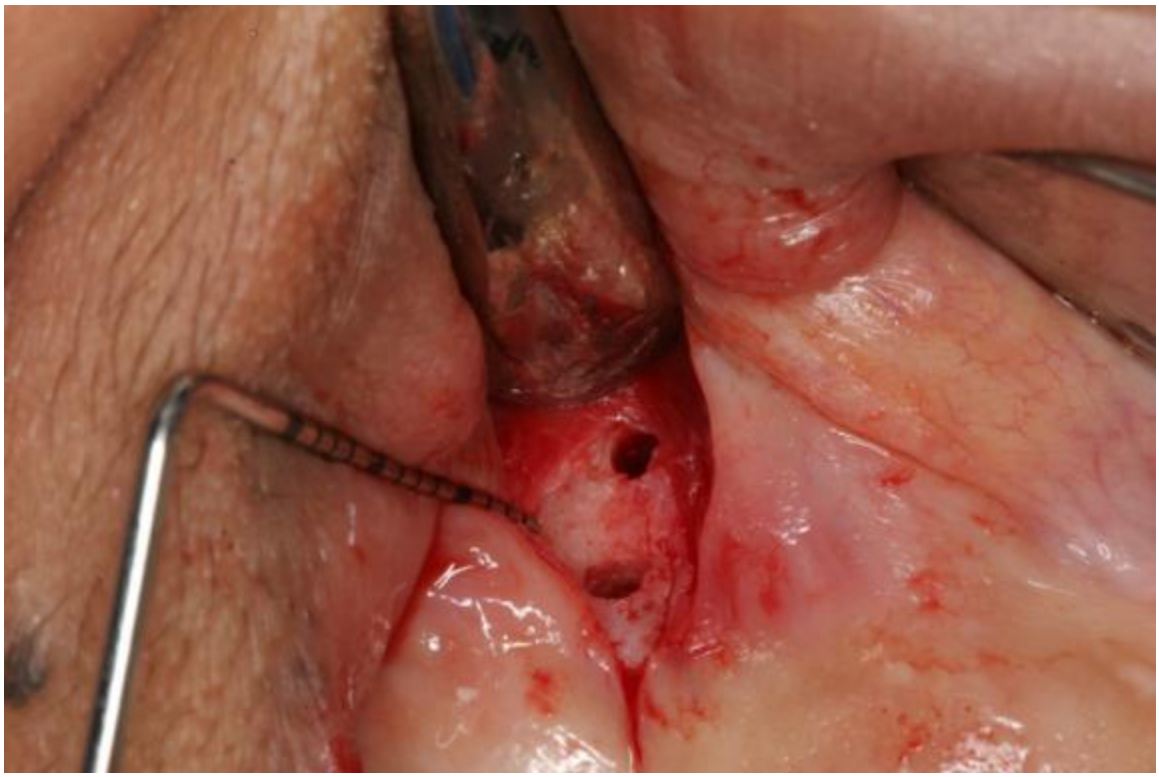


Figure 3

Prepared osteotomies. Three individual implant surgical sites were prepared in each patient. Each site would contain two osteotomies prepared to a 5 mm depth. This approach was utilized so that explantation surgeries would not disrupt healing around other implant sites.

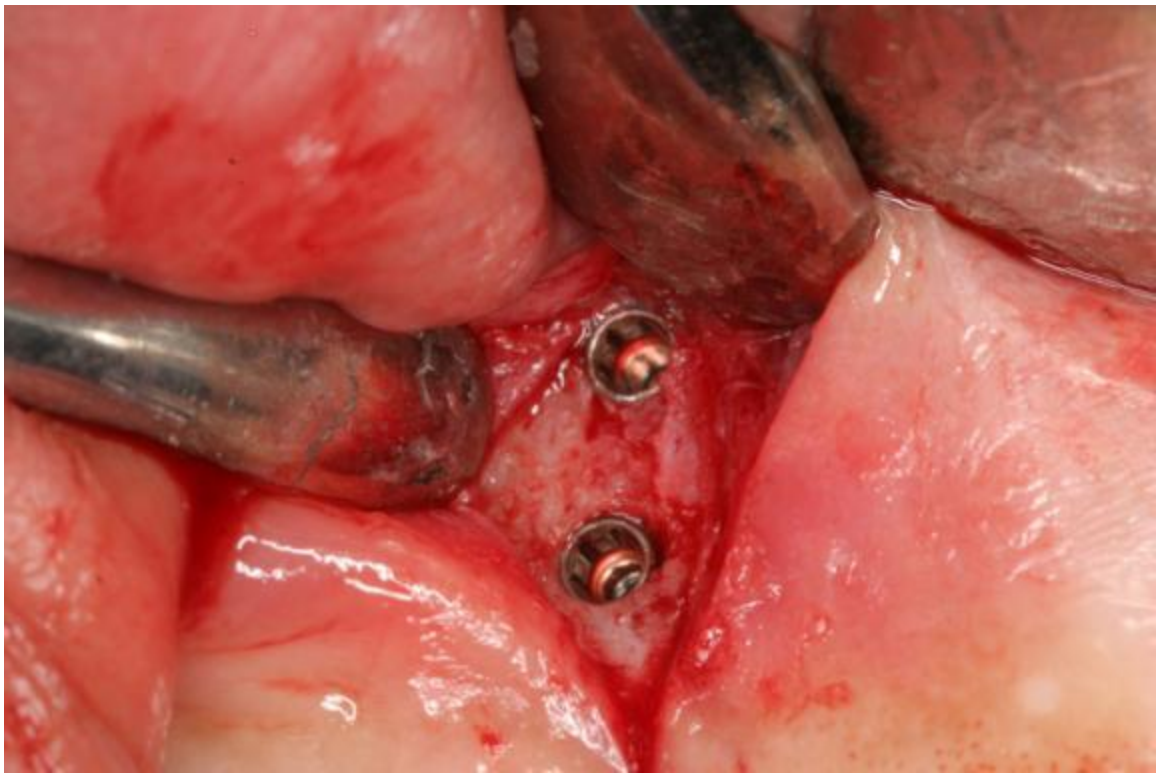


Figure 4

Experimental implants in place. Implants were placed to insure greatest intimate contact with the osteotomy site. The micron topography control implant was placed in the most superior osteotomy site. Implants were allowed to heal for twenty-four hours, seventy-two hours, and one week post insertion.

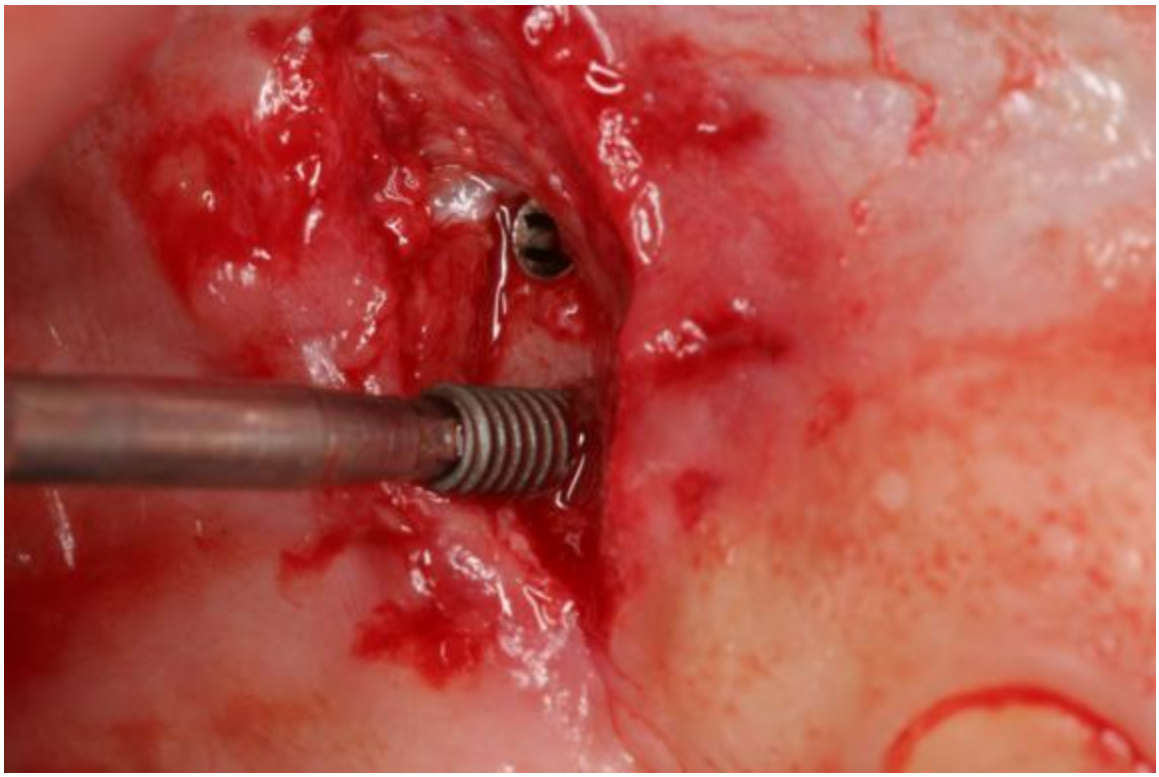
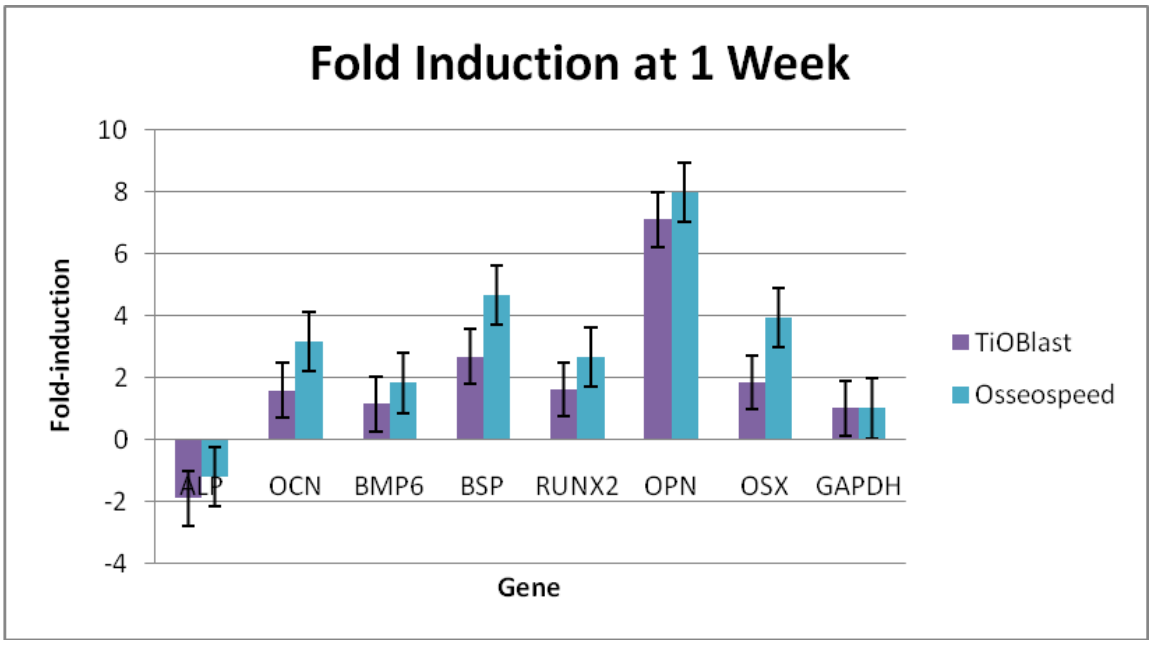


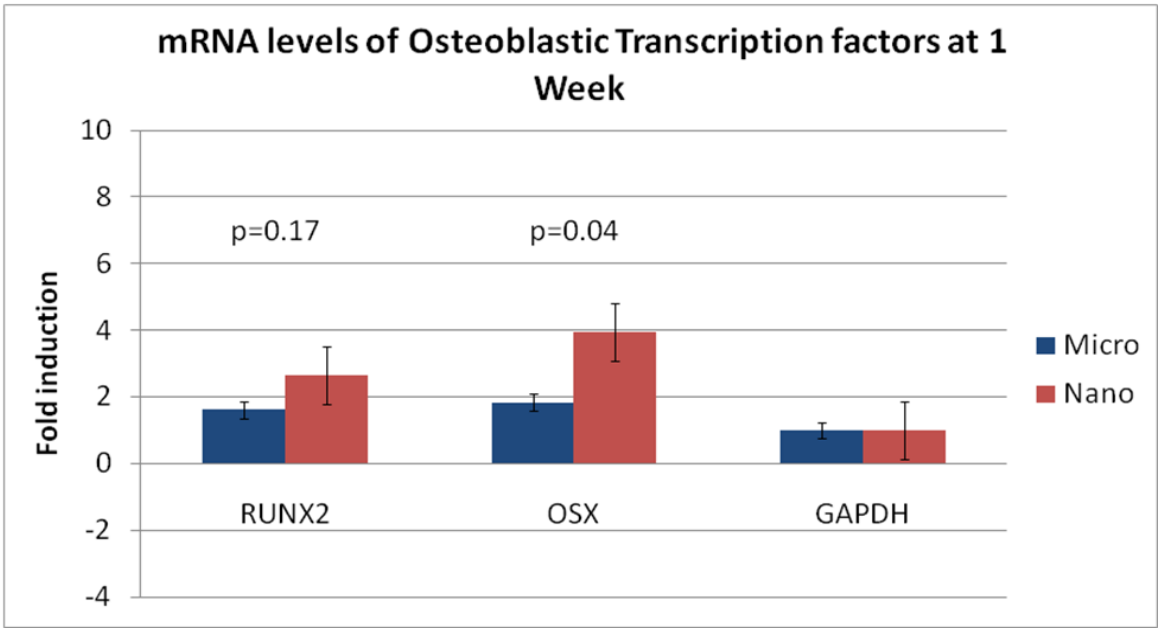
Figure 5

Explantation surgery. Implants were removed by reverse threading. This allowed the analysis of only adherent cells. The trephination of these implants may have removed additional cells not involved with the peri-implant healing as well as increase study subject morbidity. Explanted implants were immediately rinsed in neutral Phosphate Buffered Saline (PBS) to remove blood products and loosely adherent cells. Implants were then placed in Trizol Reagent to lyses cells in preparation of biochemical analysis.



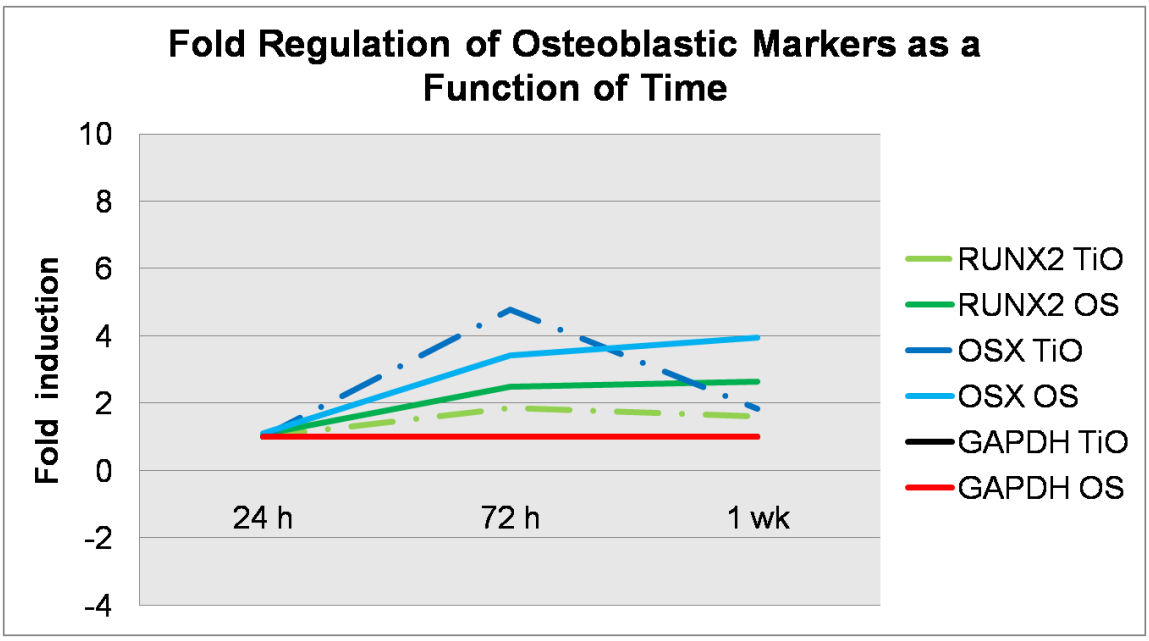

Graph 1

The graph of fold induction at one week. Real-time PCR measurement of mRNA levels of the adherent cells to cpTitanium implants. At day seven, mRNA expression levels were measured, nanotopography implants showed greater gene expression of all gene markers but these differences were not statistically significant. GAPDH was used as the control gene.



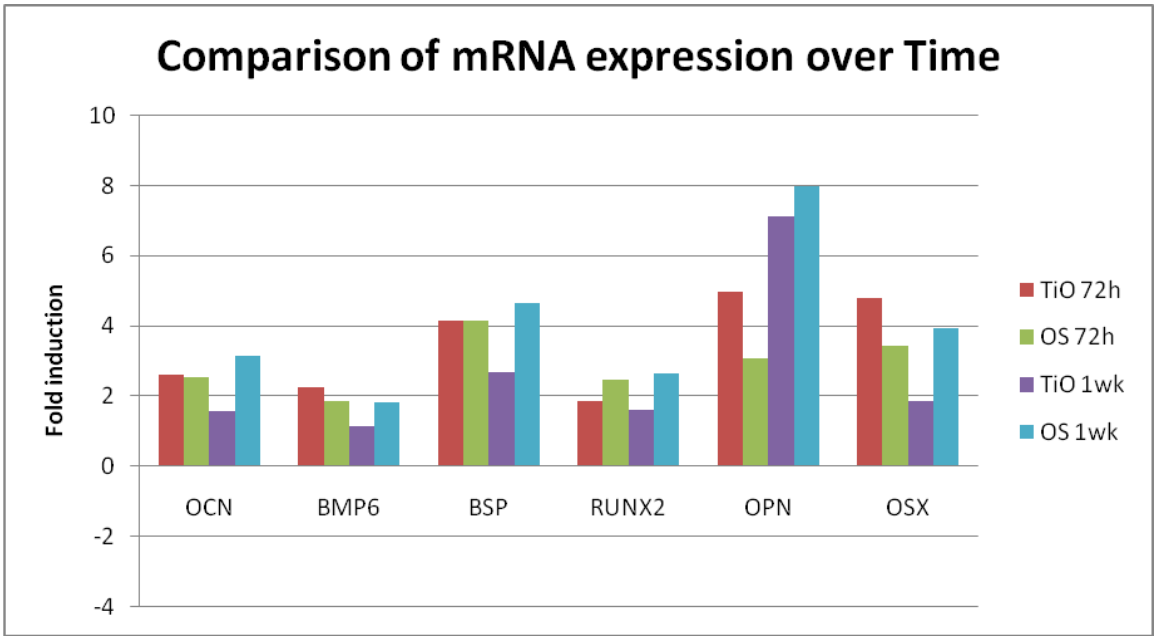

Graph 2

The graph of fold induction at one week for transcription factors. To elucidate how nanotopography surfaces may affect osteogenesis we analyzed the key transcription factors of RUNX2 and OSX. Greater expression of transcription factors were seen on nanotopographic surfaces, with OSX expression being significantly greater expression ($p=0.04$) as compared to microtopography surfaces.




Graph 3

Fold regulation as a function of time. OSX has greater expression on micron topography implants at 72 hours which precipitously fell at the 1 week mark. At one week nanotopography implants had greater expression of transcription factors than microtopography implants. There are several possibilities for this pattern including a response to pro-inflammatory markers, statistical error a result of small sample size, or an otherwise unknown biological process. Additional research would be required to elucidate a possible mechanism for this analysis.




Graph 4

The graph of mRNA expression at 72 hr and 1 week. Note that mRNA levels are greater for OCN, BMP6, and OSX at the 72 hour time point than at the 1 week point. For BMP6 and OSX, micron topography implants actually exceed mRNA levels of nanotopography implants at the 1 week mark. All micron topography mRNA levels are lower at the 1 week mark.

## WORKS CITED


Albrektsson, T. (2001). "Is surgical skill more important for clinical success than changes in implant hardware?" Clin Implant Dent Relat Res **3**(4): 174-5.

Albrektsson, T. and C. Johansson (2001). "Osteoinduction, osteoconduction and osseointegration." Eur Spine J **10 Suppl 2**: S96-101.

Albrektsson, T. and A. Wennerberg (2004). "Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them." Int J Prosthodont **17**(5): 536-43.

Albrektsson, T. and A. Wennerberg (2004). "Oral implant surfaces: Part 2--review focusing on clinical knowledge of different surfaces." Int J Prosthodont **17**(5): 544-64.

Bain, C. A. (1996). "Smoking and implant failure--benefits of a smoking cessation protocol." Int J Oral Maxillofac Implants **11**(6): 756-9.

Berry, C. C., M. J. Dalby, et al. (2006). "The interaction of human bone marrow cells with nanotopographical features in three dimensional constructs." J Biomed Mater Res A **79**(2): 431-9.

Boyle, D. L., S. Rosengren, et al. (2003). "Quantitative biomarker analysis of synovial gene expression by real-time PCR." Arthritis Res Ther **5**(6): R352-60.

Brody, S., T. Anilkumar, et al. (2006). "Characterizing nanoscale topography of the aortic heart valve basement membrane for tissue engineering heart valve scaffold design." Tissue Eng **12**(2): 413-21.

Buser, D., R. K. Schenk, et al. (1991). "Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs." J Biomed Mater Res **25**(7): 889-902.

Fiorellini, J. P., P. K. Chen, et al. (2000). "A retrospective study of dental implants in diabetic patients." Int J Periodontics Restorative Dent **20**(4): 366-73.

Guo, J., R. J. Padilla, et al. (2007). "The effect of hydrofluoric acid treatment of TiO2 grit blasted titanium implants on adherent osteoblast gene expression in vitro and in vivo." Biomaterials **28**(36): 5418-25.

Hansson, S. and M. Norton (1997). The relation between surface roughness and interfacial shear strength for bone anchored implants: A biomechanical approach...Towards optimized dental implant...

Hansson, S. and M. Norton (1999). "The relation between surface roughness and interfacial shear strength for bone-anchored implants. A mathematical model." J Biomech **32**(8): 829-36.

Ivanoff, C. J., G. Widmark, et al. (2003). "Histologic evaluation of bone response to oxidized and turned titanium micro-implants in human jawbone." Int J Oral Maxillofac Implants **18**(3): 341-8.

Komori, T. "Regulation of bone development and extracellular matrix protein genes by RUNX2." Cell Tissue Res **339**(1): 189-95.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-8.

Mendonca, G., D. B. Mendonca, et al. "The combination of micron and nanotopography by H(2)SO(4)/H(2)O(2) treatment and its effects on osteoblast-specific gene expression of hMSCs." J Biomed Mater Res A.

Mendonca, G., D. B. Mendonca, et al. (2008). "Advancing dental implant surface technology--from micron- to nanotopography." Biomaterials **29**(28): 3822-35.

Mendonca, G., D. B. Mendonca, et al. (2009). "The effects of implant surface nanoscale features on osteoblast-specific gene expression." Biomaterials **30**(25): 4053-62.

Mendonca, G., D. B. Mendonca, et al. (2009). "Nanostructured alumina-coated implant surface: effect on osteoblast-related gene expression and bone-to-implant contact in vivo." Int J Oral Maxillofac Implants **24**(2): 205-15.

Nakashima, K., X. Zhou, et al. (2002). "The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation." Cell **108**(1): 17-29.

Palin, E., H. Lui, et al. (2005). "Mimicking the nanofeatures of bone increases bone-forming cell adhesion and proliferation." Nanotechnology **16**(9): 1828-1835.

Rocci, M., A. Rocci, et al. (2008). "A comparative study of TiOblast and OsseoSpeed implants retrieved from humans." Applied Osseointegration Research **7**: 26-30.

Shalabi, M. M., A. Gortemaker, et al. (2006). "Implant surface roughness and bone healing: a systematic review." J Dent Res **85**(6): 496-500.

Sul, Y. T., C. Johansson, et al. (2005). "The bone response of oxidized bioactive and non-bioactive titanium implants." Biomaterials **26**(33): 6720-30.