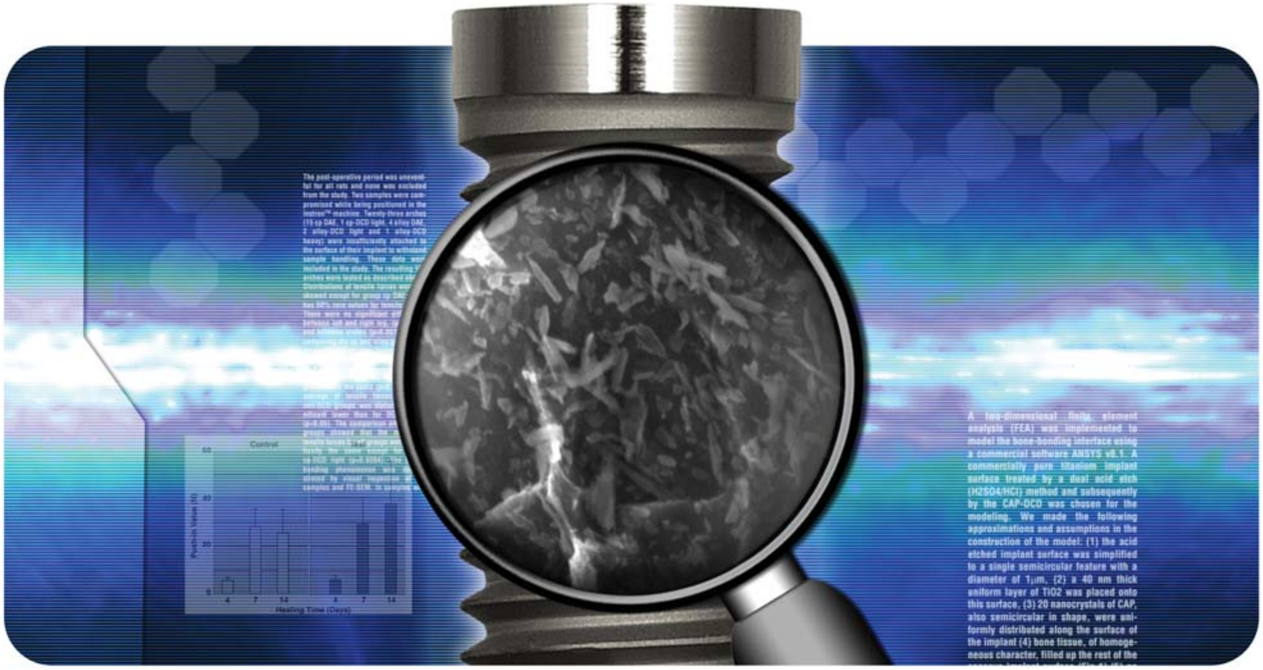


Research Dossier

The NanoTite™ Implant

NanoTite
PREVAIL® Implant

NanoTite
Tapered Implant

NanoTite
Certain® Implant

NanoTite External
Connection Implant

Steve Schiess

We are proud to present in this dossier a body of data documenting the performance of the NanoTite™ Implant.

For more than ten years we have utilized the dual-acid-etched OSSEOTITE® Surface treatment to improve our implants' success and more importantly change the way our customers are able to treat their patients. Before the launch of the OSSEOTITE Implant, a two-stage implant placement and restoration procedure requiring a four to six month submerged healing protocol was the standard approach. The clinical and preclinical studies conducted and published on the OSSEOTITE Implant provided insights as to the nature of how dental implants heal and function in general. Clinical investigators found that "early loading" in which implants were placed in a single-stage approach and put into function at eight weeks was just as successful as delayed-loading cases. The growing demand by patients and clinicians for immediately loaded restorations suggested that additional surface improvements might be of clinical benefit.

In early 2003, research efforts at the UCLA Weintraub Center brought to our attention nano-technology that we have used to further enhance the OSSEOTITE Surface. Throughout the past four years, the combined efforts of researchers and clinicians from around the globe have allowed for the accumulation of data to help characterize the effects of the new surface. Contributions from researchers at the Universities of Toronto, UCLA, D'Anunzio in Chieti, Italy and elsewhere provided the product development group at BIOMET ***3i*** *the means to validate this technology and launch it into commercial introduction as a new dental implant known as the NanoTite™ Implant.*

The following NanoTite Implant results are compelling and as much as we are interested in the nature of these results, we are more concerned with keeping a focus on the benefits we believe can be appreciated by patients and clinicians. We hope you find the studies and results presented herein of interest and helpful for considering how this new technology may benefit patient care.

Steve Schiess, President
BIOMET ***3i***

Table Of Contents

Surface Characterization Studies

Dissolution Of Discrete Calcium Phosphate Crystals From Candidate Ti-Based Implant Surfaces

Padina Pezeshki, Stanley Lugowski And John E. Davies, Faculty Of Dentistry, University Of Toronto, Toronto, ON, Institute Of Biomaterials And Biomedical Engineering, University Of Toronto, Toronto, ON

Society For Biomaterials
32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Statement of Purpose

Plasma-spraying of bone implants with CaP accelerates peri-implant healing and renders implant surfaces bone bonding. Clinical success has been achieved, but problems have also been identified due to disintegration and delamination of thick (~ 50µm) coatings.[1] Also, the associated high temperature, results in a heterogeneous coating comprising several CaP phases.[2] Thus, the solubility of coatings has been a subject of considerable study, and different fabrication methods have aimed to modify the properties that influence the CaP dissolution rate. A recent means of modifying surfaces with CaP comprises deposition of discrete crystals (DCD™) of CaP (20-100 nm) onto the metal surface. Our current study was designed to quantify the amount and dissolution kinetics of these discrete CaP crystals from dental implant surfaces and compare their behavior with commercially-available plasma sprayed CaP coated implants of a similar size. We chose an investigational pH range of 4-7 for our experiments.

Methods

DCD-treated implants (6mmØx15mmL) and plasma sprayed CaP implants (6mmØx13mmL) were supplied by BIOMET ***3i*** (Palm Beach Gardens, FL). The experiments measured (i) total amount of Ca on the surface and (ii) amount of Ca released in pH-specific solutions. All experimental equipment was initially cleaned to remove containing Ca contaminants. For determination of total Ca, each DCD-treated implant was immersed in 10 ml of 1 M Ultrex Grade HCl (pH 0) for 15 min and each plasma sprayed sample for 16 hrs (n=8). For the second part of the experiment, samples were placed in 10 ml of saline solution with fixed pH of 4, 5, 6, or 7 for 15 min (n=3). The samples were then removed and the total Ca in the remaining solution was measured using atomic absorption spectrophotometry. Furthermore, the morphology of the surfaces was qualitatively analyzed using field emission scanning electron microscopy.

Results/Discussion

The results showed that DCD-treated implants had 3 orders of magnitude less CaP than plasma sprayed implants. As DCD-treatment is not a coating, delamination is not a potential problem, and as the crystals are of the nanometer scale range, should they become detached from the implant surface they can be easily phagocytosed by cells and degraded. Dissolution per unit time showed an inverse relationship with pH. This is relevant to the initial steps of biological healing, where activated macrophages, which randomly contact the implant surface, reduce the local pH to 4. However, at normal body pH of 7.4, the dissolution of CaP from DCD implants is almost zero, while dissolution continues to occur from plasma sprayed implants. Finally, the pH-dependent dissolution of the two implant types demonstrated that the DCD had an homogeneous CaP phase while plasma sprayed samples were multi-phasic.

Conclusions

DCD-treatment provides the surface with the advantages of CaP while utilizing over 3000 times lower coverage than traditional plasma spraying. The treatment also retains a homogenous and stable phase of CaP compared to the more soluble and hence undesirable multi-phase CaP coverage achieved through plasma spraying.

References

1. Sun *et al.*, J Biomed Mater Res. 2001;58(5):570-92.
2. Yang *et al.*, J Biomaterials 2005; 26 327-337.
3. Zeng *et al.*, J Biomaterials 1999; 20 (5): 443-351

Acknowledgements

We are grateful for financial support from BIOMET ***3i*** and the Ontario Research and Development Challenge Fund (ORDCF).

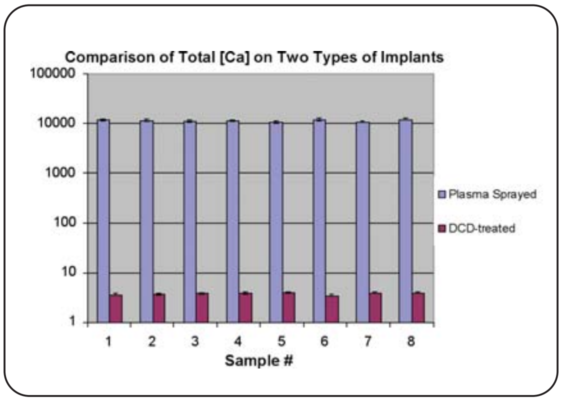


Figure 1: Comparison of total [Ca] on plasma sprayed and DCD-treated implants

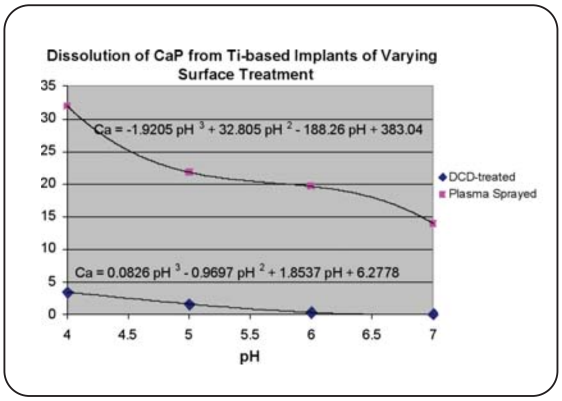


Figure 2: Dissolution trends of plasma sprayed and DCD-treated implants

Surface Area Increase Due To Discrete Crystalline Deposition™ Of Nanometer-scale CaP Crystals

Zach Suttin, Prabhu Gubbi, BIOMET ***3i****, Palm Beach Gardens, Florida, USA*

European Association For Osseointegration

15th Annual Meeting (October 5–7, 2006, Zurich, Switzerland)

Introduction

This study estimates the increase in surface area (SA) due to the deposition of nano-scale calcium phosphate (CaP) crystals onto a dual-acid-etched (DAE) implant surface.

Materials And Methods

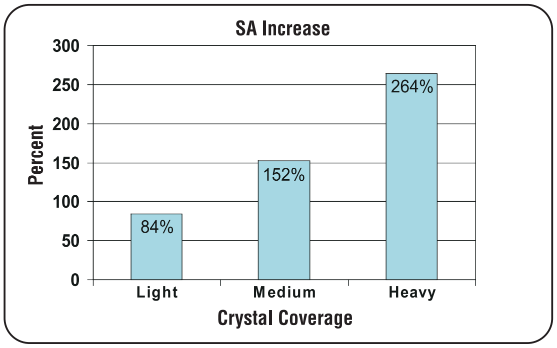
Atomic Force Microscopy (AFM) is used to quantify surface measurement parameters of surfaces with nanometer topographical characteristics. In addition to the AFM data currently being generated, this study uses theoretical 3-D modeling to estimate the SA increase due to the deposition of nano-CaP crystals onto a DAE surface. The size, shape and distribution of the nano-CaP crystals were based on the dimensions obtained from FESEM (JEOL Model 6700F) images. The crystals were modeled as ellipsoids with sizes ranging from 20-80nm on the long axis and 10-39nm on the short axis. Based on the FESEM imaging, the distributions of crystal coverage considered for the analysis were classified as light (25-30%), medium (55-60%) and heavy (65-70%).

Results And Discussion

The calculated estimates for SA increase due to nano-CaP deposition were 84%, 152% and 264% for the light, medium and heavy crystal coverage, respectively. Furthermore, compared to a machined surface, the DAE treatment increases the implant SA > 50% (data on file), and it was estimated that the increase in implant SA with the addition of nano-CaP onto the DAE surface ranges from 176% to 446%.

Conclusion

Deposition of nano-scale CaP crystals over a micron-scale DAE surface significantly increases the overall SA and topographical complexity.



Roughness Characterization Of Surface With Discrete Crystalline Deposition Of Nanometer-scale CaP Crystals

Prabhu Gubbi, Zach Suttin, Alexis Goolik, BIOMET ***3i****, Palm Beach Gardens, Florida, USA*

Academy Of Osseointegration

22nd Annual Meeting (March 8-10, 2007, San Antonio, Texas)

Introduction

The objective of this study was to characterize the surface roughness of polished titanium disks with nanometer-scale calcium phosphate (CaP) crystals deposited by a new proprietary surface treatment called Discrete Crystalline Deposition (DCD™).

Materials And Methods

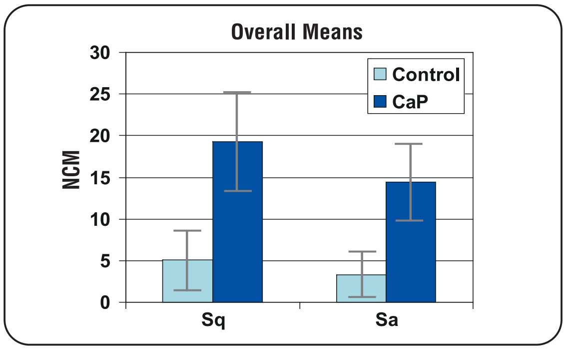
Ti (Ti-6Al-4V-ELI) disks, 20mm in diameter and 1.5mm in thickness, were machined and polished on one side to a mirror finish (Control group). A group of polished disks were deposited with nano-scale CaP crystals using the DCD process (CaP group). Atomic Force Microscopy (AFM), widely employed to quantify surface measurement parameters of surfaces with nanometer topographical characteristics, was used to obtain surface maps. Using AFM (Model MMAFM-2, Digital Instruments) in tapping mode, two disks from each group were analyzed with various spot sizes. The data were post-processed using a 3x3 low filter, with background subtraction, and line by line averaging with a 3x3 degree polynomial. The key roughness parameters obtained were Sq (root mean square variation over the surface) and Sa (absolute mean height deviation).

Results And Discussion

The calculated overall means for the Control group (N = 20 data points) were Sq = 5.04nm ± 3.62nm and Sa = 3.29nm ± 2.37nm. For the CaP group (N = 18 data points), the corresponding values were Sq = 19.3nm ± 6.4nm and Sa = 14.8nm ± 5.1nm.

Conclusion

The surface roughness characterization using AFM suggests that the deposition of nanometer-scale CaP crystals over a polished surface substantially increases the topographical complexity.



Adhesion Shear Strength Of Nanometer-scale CaP Crystals Applied By Discrete Crystalline Deposition™

Zach Suttin, Prabhu Gubbi, BIOMET ***3i****, Palm Beach Gardens, Florida, USA*

European Association For Osseointegration

15th Annual Meeting (October 5–7, 2006, Zurich, Switzerland)

Introduction

In this study, the adhesion shear strength of nano-scale calcium phosphate (CaP) crystals deposited onto dual-acid-etched (DAE) surfaces was evaluated using Atomic Force Microscopy (AFM) and was compared to the shear stress at the implant-bone interface during implant placement.

Materials And Methods

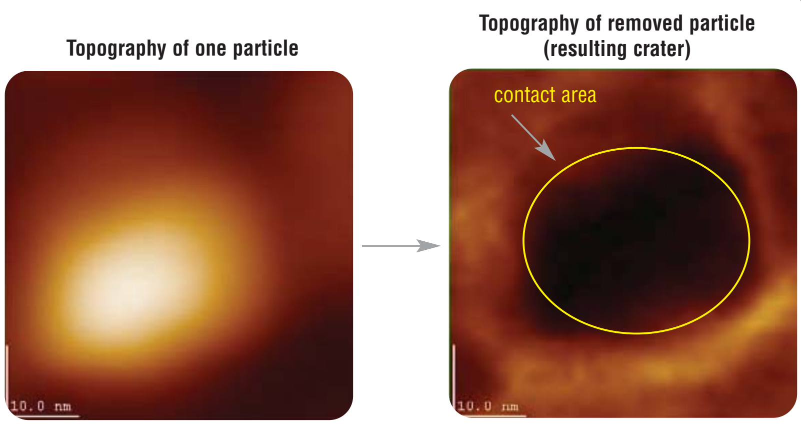
CaP crystals deposited onto CP Ti (n=30) and Ti Alloy (n=30) DAE surfaces were dislodged using contact mode AFM (Model MMAFM-2, Digital Instruments). The adhesion shear strength of each crystal-surface bond was calculated as the ratio of the maximum shear force supported by the crystal before separation to the contact area between the crystal and the surface. In a separate experiment, the torque required to place 6mm diameter implants into simulated bone (polyurethane foam of density 640kg/m^3) was measured (n=30) with a Mark-10 digital torque indicator. The average and maximum shear stresses generated at the implant-bone interface were calculated from the implant insertion torque values. For additional details, see pages 7-10.

Results And Discussion

The average crystal-surface adhesion shear strength from AFM analysis was 1.75GPa for CP Ti and 1.52GPa for Ti Alloy. The maximum torque for implant placement was 17N-Cm, which resulted in an average shear stress of 3.61x10^{-4}GPa at the implant-bone interface. The maximum shear stress, occurring at the fore threads in the apical zone, was 5.14x10^{-1}GPa.

Conclusion

The average crystal-surface adhesion shear strength was three orders of magnitude greater than the average implant-bone interface shear stress. The results indicate that the nano-scale CaP crystals deposited onto either CP Ti or Ti Alloy DAE surfaces will not dislodge during implant placement.



Shear Strength Analysis Using Contact-mode Atomic Force Microscopy (AFM)

Determination Of CaP Crystal Shear Strength Using Contact-Mode Atomic Force Microscopy (AFM)

Gajendra Shekhawat, PhD - Research Assistant Professor, Northwestern University Institute of Nanotechnology, Northwestern University
Zach Suttin , BIOMET ***3i***, *Palm Beach Gardens, Florida, USA*

Bench Testing

Abstract

In a new surface treatment process, discrete nanometer-scale calcium phosphate (CaP) crystals are bonded to an OSSEOTITE® Implant surface. The strength of the bond between the implant surface and the CaP crystals can be used as an indicator of the likelihood of the discrete crystals becoming dislodged during implant placement. The objective of this experiment was to determine the shear strength of these crystals deposited on an OSSEOTITE Implant surface using contact-mode AFM analysis.

Background

Contact-mode AFM analysis can be used to determine the adhesion strength (e.g., shear strength) of a discrete nanometer-scale particle (such as a CaP crystal) bonded to a surface. This analysis uses a nanometer length scale Silica Nitride (SiN) calibrated beam with a diamond coated probe (or tip). The probe is displacement-controlled and the equivalent force is calculated using beam mechanics. The contact-mode AFM analysis is initiated by placing the probe on a discrete particle. The beam displacement is slowly increased until the displacement load dislodges the particle. For shear strength and compressive strength analysis, the probe is moved tangential and normal to the surface on which the crystal is adhered to. Figure 1 depicts an example shear strength contact-mode AFM assay.

The results can be used to determine the shear strength of the crystal. The shear strength **(τ_s)** is a function of:

- Force **(F)**
- Contact area **(A_c)**

The shear strength can be equated as:

$$\tau_s = \frac{F}{A_c} \text{ ....equation (1)}$$

The results for the above experiment shown in (Figure 1) can be interpreted as follows:

Contact Area **(A_c)** – Since the particle appears to be approximately 20nm in diameter (relative to the scale), the contact area between the particle and the surface can, therefore, be reasonably estimated to be between $7.85e^{-17}m^2$ and $3.14e^{-16}m^2$ (based on a contact radius of 5nm to 10nm).

Shear Force **(F)** – A 150nN shear force was required to dislodge the particle.

Shear Strength **(τ_s)** – Using equation (1), the shear strength of the particle can be estimated to be between $4.7x10^{-1}$GPa and 1.91GPa. The range in shear strength is due to the estimated range of the contact area as described above.

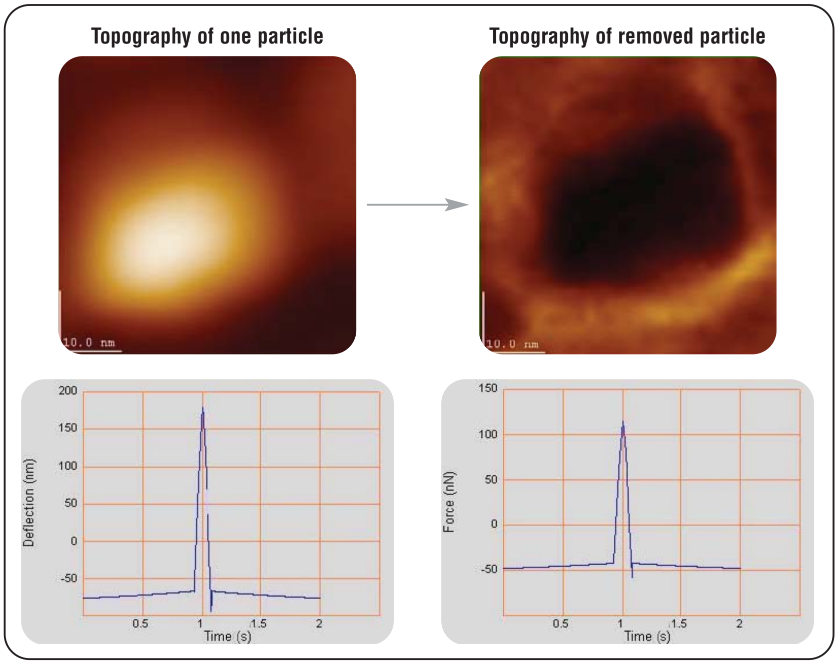


Figure 1: Shear strength contact-mode AFM assay

Materials

- Three (3) nanometer-scale CaP crystal processed CP Ti OSSEOTITE® 20mm disks (Control #4543 / Batch #115-72-B)
- Three (3) nanometer-scale CaP crystal processed Ti-Alloy OSSEOTITE 20mm disks (Control #4544 / Batch #115-72-B)
- Contact-mode AFM (Multimode PicoForce Scanning Probe Microscope)

Methods

- Shear thirty (30) crystals from the CP Ti disks using contact-mode AFM. Acquire the shear force and contact area values for each data point.
- Shear thirty (30) crystals from the Ti-Alloy disks contact-mode AFM. Acquire the shear force and contact area values for each data point.

Although the main objective of this assay was to determine the shear strength of the nanometer size CaP crystals, an abbreviated compressive strength assay was subsequently conducted. The purpose of this secondary experiment was to verify that the compressive strength of the crystals exceeded the shear strength of the crystals bonded to the OSSEOTITE Surface. Four crystals, two from each substrate type, were compressively loaded with the probe until they fractured. The compressive force values required to fracture the crystals ranged from 3.8μN to 6.60x10^{2}μN. The 3.8μN value is more than one order of magnitude greater than the shear force values required to shear the crystals from the substrate.

The experimental analysis was conducted by Dr. Gajendra Shekhawat at Northwestern University's Atomic and Nanoscale Characterization Experimental Center (NUANCE).

Results

Table 1 lists the results for the contact-mode AFM analysis. The force values, which are a function of beam displacement, are output by the AFM software. The contact area values are measured with the AFM following the removal of each crystal. The shear strength was calculated using equation (1).

The mean, standard deviation, low and high shear strength values for the nanometer-scale CaP crystals bonded to both types of OSSEOTITE substrates are shown in Table 2. The crystals on the CP Ti substrate had an average strength 15% higher than the crystals on the Ti Alloy substrate.

Table 1: AFM Analysis Results

	Ti Alloy			CP Ti		
Crystal No.	Force (nN)	Area (nm^2)	Shear Strength (GPa)	Force (nN)	Area (nm^2)	Shear Strength (GPa)
1	128	137.2	0.93	241	114.3	2.11
2	166	162.6	1.02	242	141.4	1.71
3	228	128.2	1.78	236	137.6	1.72
4	167	143.5	1.16	243	142.3	1.71
5	188	141.6	1.33	241	167.6	1.44
6	232	118.4	1.96	242	171.4	1.41
7	187	118.6	1.58	238	154.6	1.54
8	202	108.4	1.86	240	124.7	1.92
9	224	141.7	1.58	231	148.9	1.55
10	230	148.4	1.55	242	179.4	1.35
11	222	146.6	1.51	243	119.6	2.03
12	207	133.5	1.55	236	122.4	1.93
13	206	145.5	1.42	241	113.4	2.13
14	222	115.6	1.92	237	121.4	1.95
15	201	129.6	1.55	242	136.4	1.77
16	191	123.3	1.55	252	138.5	1.82
17	218	112.7	1.93	237	142.2	1.67
18	188	118.2	1.59	256	120.4	2.13
19	187	117.2	1.60	254	175.4	1.45
20	193	113.4	1.70	253	172.2	1.47
21	175	132.6	1.32	296	117.4	2.52
22	200	115.6	1.73	258	126.4	2.04
23	184	106.6	1.73	269	132.4	2.03
24	196	114.7	1.71	241	129.6	1.86
25	209	129.5	1.61	222	132.7	1.67
26	219	149.5	1.46	249	126.4	1.97
27	232	131.2	1.77	221	156.4	1.41
28	204	166.8	1.22	219	144	1.52
29	222	225	0.99	238	162	1.47
30	236	258.8	0.91	279	242	1.15

Table 2: Shear Strength Statistics From Table 1

	Shear Strength (GPa)			
Substrate	Average	Standard Deviation	Low	High
Ti Alloy	1.52	0.29	0.91	1.96
CP Ti	1.75	0.3	1.15	2.52

Qualitative Evaluation Of Crystal Adhesion During Implant Placement In Simulated Bone Medium

Zach Suttin, BIOMET ***3i***, *Palm Beach Gardens, Florida, USA*

Bench Testing

Abstract

The implant-bone interface experiences shear stress during implant placement. The objective of this test was to qualitatively measure whether or not nanometer-scale CaP crystals discretely bonded to OSSEOTITE® Implants would dislodge during placement and removal in a simulated bone material. Ti- Alloy and CP Ti OSSEOTITE Implants with discretely bonded nanometer-scale CaP crystals were placed in and removed from a simulated bone medium. The implants were then imaged with a field emission scanning electron microscope (FESEM) to determine if the nanometer-scale CaP crystals had become dislodged. Based on the imaging results, it is evident that the nanometer-scale CaP crystals did not become dislodged during implant placement and removal.

Materials

- Six (6) nanometer-scale CaP crystal processed Ti-Alloy 4mm TG FOSS CaP implants (Control #P-84 / Batch #115-68-B)
- Six (6) nanometer-scale CaP crystal processed CP Ti 4mm TG FOSS CaP Implants (Control #P-83 / Batch #115-68-B)
- 3740 LAST-A-FOAM®
- Medium stiffness toothbrush

Methods

The two groups of six (6) implants were used in the experiment as follows:

1) Control
2) Brush
3) Polymer
4) Polymer + Brush (1)
5) Polymer + Brush (2)
6) Polymer + Brush (3)

Two of the implants in each group (1 & 2) were not screwed into the simulated bone medium. The number (1) implant was used as a control and the number (2) implant was used to determine if the toothbrush alone would remove the CaP crystals. Four of the implants from each group (3, 4, 5 & 6) were screwed into and removed from the simulated bone medium. Implants (4), (5) and (6) were then brushed to try and remove the simulated bone medium (polymeric material). The number (3) implant was not brushed off so it could be used for comparison to the other implants.

STEP 1: Eight (8) blind holes in the 3740 LAST-A-FOAM were drilled using a 3.25mm twist drill.

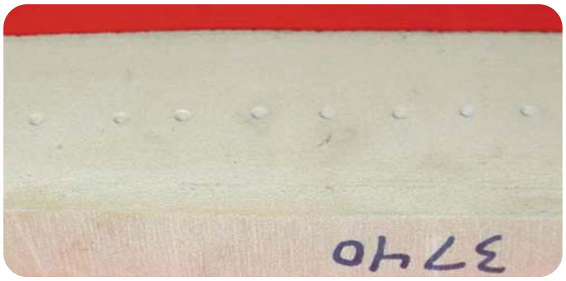


Figure 1: 3740 LAST-A-FOAM with eight (8) 3.25mm blind holes

STEP 2: Four (4) implants from each group were screwed into one of the blind holes and then removed.

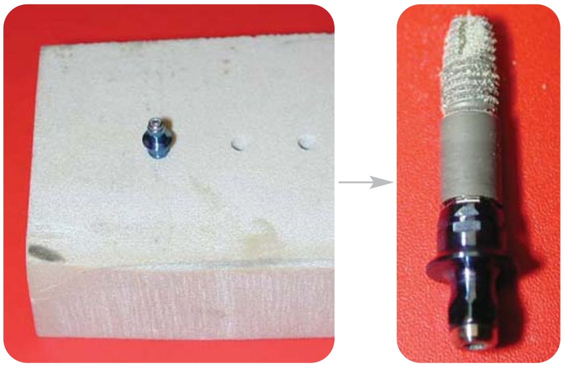


Figure 2: An implant placed in and then removed from a blind hole

STEP 3: Three (3) of the four (4) implants from each group which were screwed into the bone were lightly brushed to remove the polymeric debris.

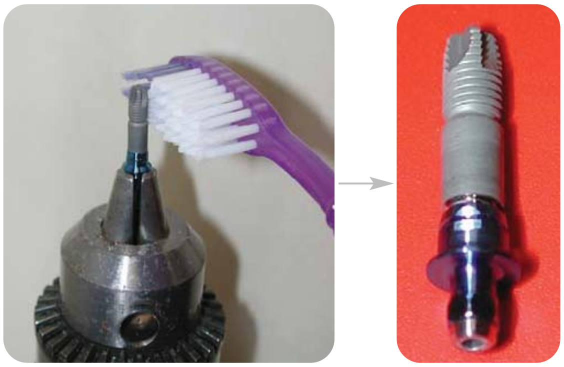


Figure 3: An implant placed in a fixture for brushing and then removed

STEP 4: The implants were then imaged using a FESEM at the apical (in red) and coronal (in green) aspects. The images were taken on surfaces at a 45 degree angle relative to the electron beam direction. The FESEM imaging was performed by Materials Analytical Services, Inc. in Raleigh, NC.

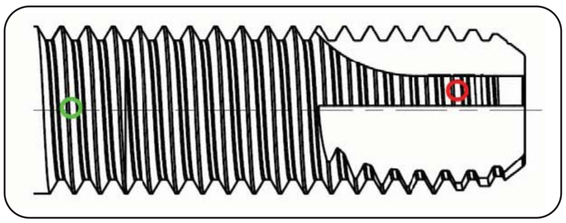
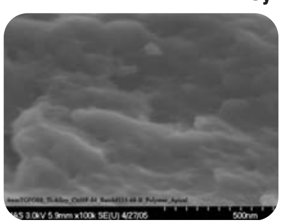


Figure 4: Location of acquired FESEM images

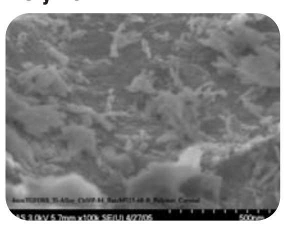
Results

Below is an example of a 100kX image for the apical and coronal aspects of one of the twelve (12) implants:

Ti-Alloy + Polymer



Apical



Coronal

Observations

- The "control" implant images show nanometer-scale CaP crystals in both the apical and coronal aspects.
- The "brush" implant images show nanometer-scale CaP crystals in both the apical and coronal aspects. The brushing, therefore, did not remove the nanometer-scale CaP crystals.
- The "polymer" implant images show nanometer-scale CaP crystals at the coronal aspect and not at the apical aspect. The polymer, therefore, agglomerated at the apical aspect during implant placement (meaning that the nanometer-scale CaP crystals sheared/collected the polymer). The nanometer-scale CaP crystals in the coronal aspect did not shear the polymeric material as much as in the apical aspect since the threads in the coronal aspect are just following the geometry created by the apical threads versus shearing the simulated bone medium.
- The "polymer + brush" implant images were similar to the "polymer" implant images. One difference is that in the "CP Ti polymer + brush (1)" images the nanometer-scale CaP crystals in both the apical and coronal aspects can be seen. It was anticipated that this would be the case for all "polymer + brush" implants, but it turned out to be difficult to remove the polymer with the brush in most instances. Another difference is that polymeric material can be seen in the "Ti-Alloy + Polymer + Brush (1)" coronal image. So in this one case some polymeric material agglomerated in the coronal aspect of the implant (as opposed to just the apical aspect) as well.

Conclusion

This assay indicates qualitatively that nanometer-scale CaP crystals discretely bonded to OSSEOTITE® Implant surfaces will not become dislodged during implant placement. Although the nanometer-scale CaP crystals could not be seen on the apical aspect post screw in/out, it is reasonable to assume that the crystals have been covered by the polymer (instead of being sheared off by the polymer) in this area. This argument is supported by considering the image of the apical aspect from the "CP Ti polymer + brush (1)" implant.

Modeling Interfacial Shear Strength At A CAP-Modified Titanium And Bone

Chunpo Pan, Jason Chau, Vanessa C. Mendes, Craig A. Simmons And John E. Davies, Institute Of Biomaterials And Biomedical Engineering[1], Faculty Of Dentistry[2], University Of Toronto, Canada

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Statement of Purpose

An interfacial bond, which exceeds the cohesive strength of either bone tissue or implant, characterizes the bone bonding phenomenon attributed to calcium phosphate (CAP) materials but not to metals.[1] However, CAP plasma-sprayed metallic implants have also demonstrated clinical problems of inflammatory response and implant failure under loading.[2] For this reason, CAP-coated surfaces have been largely replaced by microtextured metallic surfaces. A new technology has been recently developed, comprising the Discrete Crystalline Deposition™ (DCD™) of calcium phosphate (CAP) nanoparticles (20-80nm nominal size) on the surface of a microtextured titanium implant. We have shown such small CAP nanofeatures render titanium implants bone-bonding.[3] We have also shown bone-bonding as a mechanical interlock rather than a chemical phenomenon.[3] For this reason, we designed a mathematical model to address the question: Can the bone-bonding phenomenon be achieved exclusively by micro-mechanical interlocking mechanism?

Methods

A two-dimensional finite element analysis (FEA) was implemented to model the bone-bonding interface using a commercial software ANSYS v8.1. A commercially pure titanium implant surface treated by a dual acid etch (H_2SO_4/HCl) method and subsequently by the CAP-DCD was chosen for the modeling. We made the following approximations and assumptions in the construction of the model: (1) the acid etched implant surface was simplified to a single semicircular feature with a diameter of 1µm, (2) a 40 nm thick uniform layer of TiO_2 was placed onto this surface, (3) 20 nanocrystals of CAP, also semicircular in shape, were uniformly distributed along the surface of the implant (4) bone tissue, of homogeneous character, filled up the rest of the concave implant surface (Fig.1) (5) no bonding or adhesion was assumed at the implant/bone interface, and (6) the TiO_2/bone and CAP/bone interfaces were modeled as frictionless contact surfaces by surface-to-surface contact elements (CONT172/TARGET169) with bone as the contact surface and TiO_2 and CAP as the target surfaces. The model was designed to predict whether failure of the bone or failure of the TiO_2/CAP interface would occur for a given tensile load. Failure of bone was assumed to occur if the local maximum equivalent von Mises stress exceeded the failure strength of 1.7 MPa measured in our in vivo experiment. Failure of the TiO_2/CAP interface was assumed to occur if the equivalent von Mises stress in the interface exceeded the average shear strength of 1.75 GPa. The interface stress was calculated as the average of the modal stress for the TiO_2 and CAP at the interface. The model was analyzed (1) by increasing applied loads until the failure criterion for bone or the TiO_2/CAP interface was met and (2) by maintaining the failure criterion for bone while decreasing failure criterion for the interface such that the interface would fail under applied load.

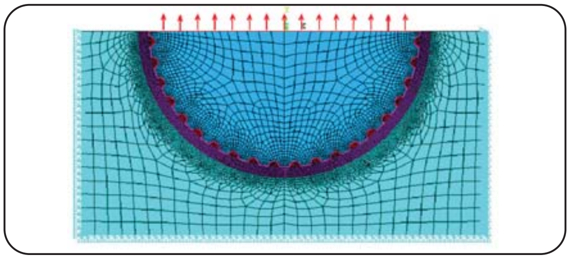


Figure 1: ANSYS model with meshing and uniformly distributed load pulling upwards.

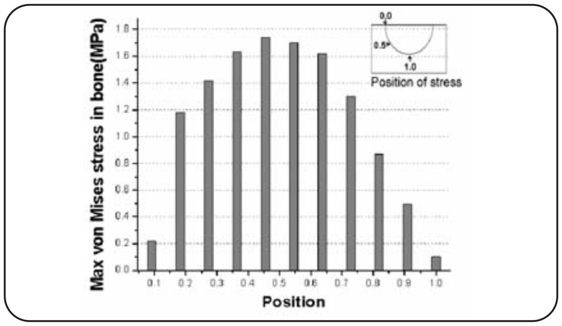


Figure 2: Stress distribution in bone at the failure (load = 44 KPa, E_{bone} = 17 GPa)

Results/Discussion

The load was determined to be 44 KPa when the maximum equivalent von Mises stress inside the bone was measured to be 1.7 MPa (Fig. 2), and that on the TiO_2/CAP interface was measured to be 1.02 MPa. This shows that the maximum von Mises stress will reach the failure criterion for the bone (1.7 MPa) before that for the TiO_2/CAP interface (1.75 GPa), and thus the fracture will first occur in the bone. If we maintain the load and decrease the strength of the TiO_2/CAP interface to 1.02 MPa, which is 3 orders of magnitude lower than reported previously, the bone will remain intact and the TiO_2/CAP interface will fail.

Conclusions

The model can withstand 44 KPa pressure due to mechanical interlocking, and shows that the bone-bonding phenomenon can be achieved exclusively by micro-mechanical interdigitation. The results also indicate that the bone will fail before the TiO_2/CAP interface, which is in accordance to our in vivo experimental results.

References

1. Hench & Wilson. Science 1984; 226:630-636.
2. Geesink et al. J Bone Jt Surg. 1992; 74:534-547
3. Mendes & Davies (2006). 25th Meeting Canadian Biomaterials Society.

Acknowledgements

BIOMET ***3i***, ORDCF, NSERC and CIHR.

Chemical And X-ray Diffraction Analyses Of Calcium Phosphate Used For Discrete Crystalline Deposition™

*Renée M. Stach, DDS, Prabhu Gubbi, PhD And Zach Suttin, BIOMET **3i**, Palm Beach Gardens, FL, USA*

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Statement of Purpose

NanoTite™ (BIOMET ***3i***, Inc., Palm Beach Gardens, FL, USA) is an implant surface modification featuring discrete crystalline depositions (DCD™) of nanometer-scale calcium phosphate (CaP) particles processed by sol gel application over the microtopography of dual acid-etched titanium alloy (OSSEOTITE®, BIOMET ***3i***, Inc.). The two-part objective of this study is (1) to confirm that the CaP particles in the colloidal solution used for the DCD process have a predetermined crystallinity and chemistry that are not altered during the application process to the titanium surface and (2) to qualitatively assess the size and shape of the CaP particles deposited on the implant surface using Field Emission Scanning Electron Microscopy (FESEM).

Methods

The X-ray Diffraction (XRD) analysis was performed using a Scintag XDS2000 diffractometer (Scintag, Inc., CA, USA) on the CaP powder used as a raw material in preparing the colloidal solution. The size of the CaP nanometer-scale particles in the colloidal solution ranges from 20nm to 100nm. The Certificate of Analysis from the manufacturer of the CaP powder states that the Ca/P ratio is 1.6. XRD was also carried out on a CaP powder sample which was obtained by drying the colloidal solution used in the DCD process. The crystallinity of the samples were identified through comparison with the built-in JCPDS powder diffraction database. An FESEM, model JEOL JSM-6700F (JEOL USA, Inc, Peabody, MA) was used to obtain high resolution imaging on the NanoTite implant surface in order to visualize the nanometer-scale CaP crystals.

Results/Discussion

The XRD analyses for both the source material sample used for preparing the colloidal solution and for the dried CaP sample that had been used in the DCD process show 100% crystallinity within the detection limits of the instrumentation and no amorphous content was detected. This result indicates that there was no change in the crystallinity due to the DCD process and therefore the chemistry of the CaP particles was not altered during the sol gel application to the titanium oxide surface layer. Figure 1 shows the XRD pattern for the dried CaP powder sample to be purely crystalline in nature.

The FESEMs of the implant surface show discrete depositions of CaP crystals. The size, shape and structure of the CaP crystals visually appear to be in the size range from 20nm to 100nm which is in agreement with the Certificate of Analysis from the manufacturer of raw CaP powder. Figure 2 shows a representative FESEM image (30,000X) of the implant surface.

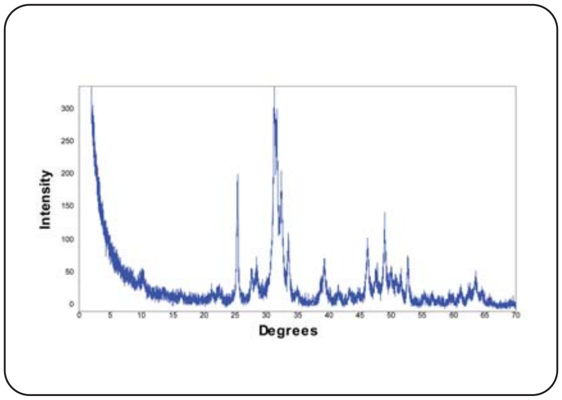


Figure 1: XRD Pattern For Dried CaP Powder Sample Which Shows It to be Purely Crystalline

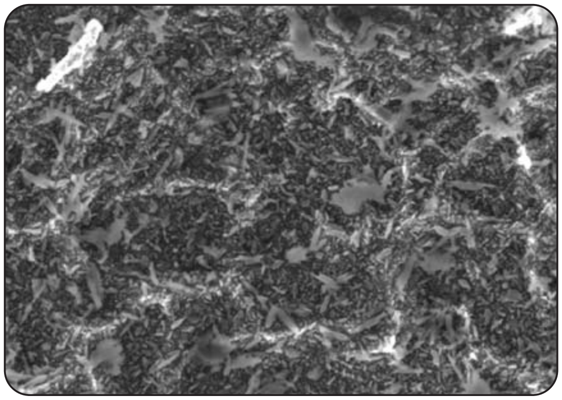


Figure 2: High Magnification FESEM Image of Implant Surface Showing Size and Shape of CaP Crystals

Conclusions

The crystallinity of the CaP particles in the raw material and the dried sample obtained from the colloidal solution used in the discrete crystalline deposition process was confirmed by the x-ray diffraction analysis to be purely crystalline in nature. This verifies that there was no change to the CaP crystallinity before and after the deposition process. The qualitative analyses of the implant surface, visualized with high resolution FESEM imaging showed that the CaP crystal size remained unchanged. By incorporating highly crystalline CaP crystals into a nano-scale textured surface, the biological benefits of hydroxyapatite and the osseogenic potential of surfaces with nanotopographical features can be realized *in vivo.*

Hydrophobic/Hydrophilic Characteristic Of Titanium Surfaces: Machined, Dual Acid Etched (OSSEOTITE®), And Dual Acid Etched With Nanometer-Scale CaP (NanoTite ™)

*Prabhu Gubbi, Ross Towse, Bruce Berckmans, BIOMET **3i**, Palm Beach Gardens, FL, USA.*

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Introduction

Contact angle, reported in degrees, is a measure of the wetting of a solid surface by a liquid. The objective of this study was to measure the static contact angle made by liquid media on various titanium surfaces and determine whether a given surface was hydrophobic or hydrophilic.

Materials and Methods

Custom-designed circular disks, 20mm in diameter and 1.5mm thick, were manufactured from commercially pure titanium (CP Ti) and Ti-6Al-4V-ELI alloy (Ti Alloy). Three groups of disks from both CP Ti and Ti Alloy were used: machined, dual-acid etched (DAE, proprietary to BIOMET ***3i***), and nanometerscale calcium phosphate hydroxide (nano-CaP) crystals deposited by a new surface treatment called Discrete Crystalline Deposition™ (DCD™, proprietary to BIOMET ***3i***). Three disks from each group were evaluated for hydrophilic or hydrophobic behavior with de-ionized (DI) water, bovine blood with citrate, and bovine blood with ACD-A. The contact angle was measured using a MD-OCA contact angle meter (Future Digital Scientific, NYC, NY) using SCA20 software (Dataphysics Gmbh, Germany) running on a desktop PC. The Sessile Drop method was used for recording the video of the interaction of the droplet with the surface for 20 seconds at the rate of 12.5 frames per second. The video was then analyzed for calculating static contact angle using the Young - Laplace method. Five readings were taken on each disk.

Results & Discussion

The static contact angle is mainly affected by two factors, surface topography or morphology and surface chemistry. In the current study, the effect of both surface chemistry (CP Ti vs. Ti Alloy with and without nano-CaP) and surface topography (machined vs. DAE) was examined. Table 1 shows the mean contact angle for various surfaces with DI water.

Figure 1 shows graphically the data presented in Table 1. The surface can be hydrophobic or hydrophilic/wettable depending on the criteria used for defining those terms. Low values of contact angle indicate that the liquid spreads or wets well, while high values indicate poor wetting. A zero contact angle represents complete wetting. In general, a surface exhibiting contact angle <90° is hydrophilic and the one exhibiting contact angle >90° is hydrophobic (Bico J, Thiele U, Quere D, Coll & Surf A: 206, 2002, 41-46). It can be seen from Fig. 1 that the machined surface (both CP Ti and Ti Alloy) and DAE Ti Alloy were hydrophilic whereas DAE CP Ti and the surface with nano-CaP (both CP Ti and Ti Alloy) were hydrophobic.

Conclusions

Hydrophobic and or hydrophilic characteristics of various titanium surfaces with varying surface topography and surface chemistry were determined by measuring static contact angle. The evaluation of the various surfaces indicates a clear correlation between the complexity of the surface topography and its hydrophilic or hydrophobic nature; the increase in surface complexity had a direct effect on rendering the surface increasingly hydrophobic.

Table 1: Contact Angle Measurements on Various Titanium Surfaces Using DI Water

Disk Type	*CP Ti*	*Ti Alloy*
Machined	81 ± 2.4	72.5 ± 1.5
OSSEOTITE	93.1 ± 2.9	71.8 ± 4.3
Nano HA	92.6 ± 5.0	121.9 ± 3.6

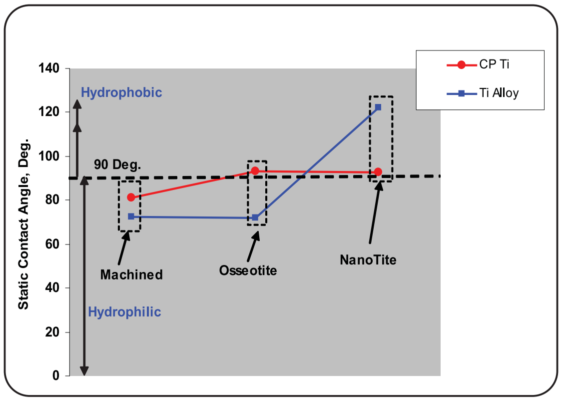


Figure 1: Graphical Representation of Contact Angle Data

Qualitative And Quantitative Analyses Of NanoTite™ Surfaced Implants

*Prabhu Gubbi, Ross Towse , BIOMET **3i**, Palm Beach Gardens, FL, USA.*

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Introduction

The objective of this study was to characterize the surface of experimental implants treated with a new proprietary surface treatment called Discrete Crystalline Deposition™ (DCD™) to deposit nanometer-scale calcium phosphate hydroxide (nano-CaP) crystals to obtain the resulting surface called NanoTite. Field Emission Scanning Electron Microscope (FESEM) was used to obtain high resolution images of the surface to visualize the size, shape and distribution of the nano-CaP crystals, and standardized software was used for quantitative analysis of crystal coverage.

Materials and Methods

Custom-designed rectangular implants, 6.0 mm x 4.0 mm x 1.5 mm, intended for antero-posterior placement into the distal metaphyses of Wistor rat femora, were manufactured from Ti-6Al-4V-ELI alloy. The implants were dual-acid etched (DAE, known as OSSEOTITE® which is proprietary to BIOMET ***3i***) and were then deposited with nano-CaP crystals by the DCD process, which is a sol-gel based deposition technique. The particle size of the nano-CaP crystals used as a raw material in the DCD process ranged from 20nm to 70nm. By controlling the various process variables, it was possible to achieve different categories of coverage, classified as light, medium, and heavy. An FESEM, model JEOL JSM-6700F (JEOL USA Inc, Peabody, MA), was used to obtain high resolution imaging of the implant surface in order to visualize the size, shape and distribution of the nano-CaP crystals. The quantitative analysis of the FESEM images was carried out to estimate the surface area coverage of the nano-CaP crystals using Scandium software (Soft Imaging System Corp., Lakewood, CO).

Results & Discussion

From the analysis of high resolution images obtained by FESEM, it was determined that the particle size (of 20nm to 70nm) did not change significantly from the raw material and the particles retained their crystalline nature after processing. Figure 1 shows the three categories of particle coverage as seen in FESEM. The quantitative area coverage was calculated using Scandium software where the nano-CaP particles were color-coded as phases and calculates the area covered by the phases. Light, medium, and heavy categories exhibited 25-30%, 55-60%, and 80-85% area coverage, respectively. Figure 2 shows the nano-CaP particles color-coded in Scandium software for area coverage calculation.

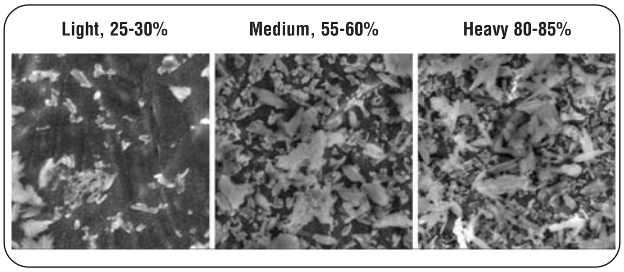


Figure 1: Field Emission SEM Images Showing Size, Shape and Distribution of Nanometer-Scale CaP Crystals with Light, Medium and Heavy Particle Coverages on DAE Surface (1 µm x 1 µm sample area at 30,000x)

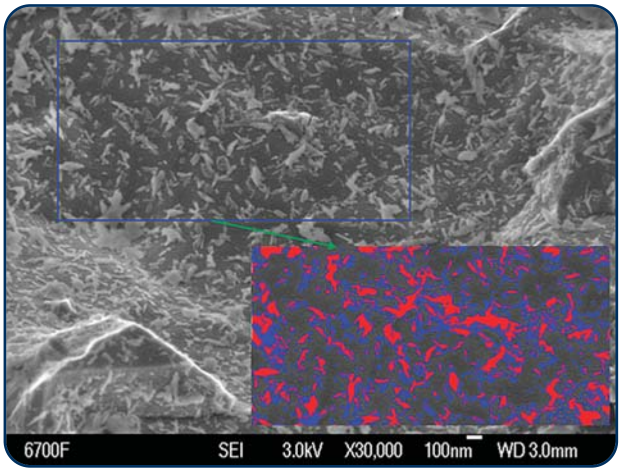


Figure 2: Phase Color-coding in Scandium Software Separates Out Nanometer-Scale-CaP Particles as Red-Blue Phase

Conclusions

The analysis of size, shape, and distribution of the particles, based on high resolution images obtained from Field emission SEM, was useful to characterize the DAE implant surface deposited with nano-CaP particles. The Scandium software was used for quantifying the light, medium and heavy surface area coverage of the nano-CaP particles.

Implants Treated With Discrete Crystalline Depositions Of Nanometer-Scale Calcium Phosphate Crystals Enhance Early Implant-Bone Fixation In A Rat Femur Push-In Model

Ichiro Nishimura, Audrey Lin, Chiachien Jake Wang, James Kelly, UCLA School Of Dentistry, The Weintraub Center For Reconstructive Biotechnology And Division Of Advanced Prosthodontics, Biomaterials And Hospital Dentistry, Los Angeles, CA, USA

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Statement of Purpose

The topography and biochemical properties of titanium implant surfaces influence the rate and extent of adherent de novo bone formation. This study uses a rat femur push-in model to demonstrate early bone fixation of implants treated with discrete crystalline depositions (DCD™) of nanometer-scale calcium phosphate crystals added to a dual acid-etched (DAE) surface.

Materials and Methods

Cylindrical miniature Ti6V4Al implants, 1mm (D) x 2mm (L), were modified with the dual acid-etched (DAE) surface treatment (OSSEOTITE®, BIOMET ***3i***, Palm Beach Gardens, FL). Test implant surfaces were additionally treated with DCD of nanometer-scale CaP (NanoTite™). The implant surfaces were examined by SMM, EDS, and SEM. Each of 24 male Sprague-Dawley rats received one Test implant in the distal end of one femur and one Control implant in the other femur. Animals were divided into groups and sacrificed after 4, 7 and 14 days of healing. The femur-implant specimens were harvested and embedded in resin blocks. An Instron® equipped with a custom-made stainless steel pushing rod was applied to the implant to determine the peak push-in force at which the implant detached from bone.[1,2]

Results/Discussion

SMM revealed that the underlying microtopography of the DAE surface was indistinguishable on both types on implants. EDS measurements at different areas indicated that the DCD implant surface chemistry was uniformly modified with Ca and P elements. High-resolution SEM revealed the size of the depositions of calcium phosphate crystals to the Ti6V4Al surface to be 20-40 nm. Mean peak push-in forces at 4 (n=7), 7 (n=7) and 14 (n=10) days for Test implants were 5.86 ± 1.82N, 29.04 ± 10.95N, and 37.48 ± 17.58N, respectively, and for Control implants were 5.54 ± 1.27N, 27.98 ± 7.53N, and 25.35 ± 9.87N, respectively ($P<0.05$ at day 14) (Figure 1). Both implant groups exhibited a substantial increase of mechanical resistance from day 4 to day 7. From day 7 to day 14, the peak push-in value for the Control group stabilized, whereas the peak push-in values for the Test group increased. The time-course pattern of early implant fixation process appeared to be different between Test and Control implants.

Conclusion

This biomechanical model demonstrates a significant increase in bone fixation for Test implants at 14 days, and suggests that while the DCD surface treatment did not alter the predisposing surface microtopography of the DAE implant substrate, the nanometer-scale calcium phosphate crystals appear to affect early implant fixation processes by a potentially unique mechanism.

(This study was supported by BIOMET ***3i*** and was conducted in part in a facility constructed with support from NIH/NCRR Grant C06RR014529.)

References

1. Ogawa, T. et al. Biomedical evaluation of osseous implants having different surface topographies in rats. J Dent Res 79, 1857-63 (2000)
2. Ozawa, S. et al. Ovariectomy hinders the early stage of bone implant integration: histomorphometic, biomechanical, and molecular analyses. Bone 30, 137-43 (2002).

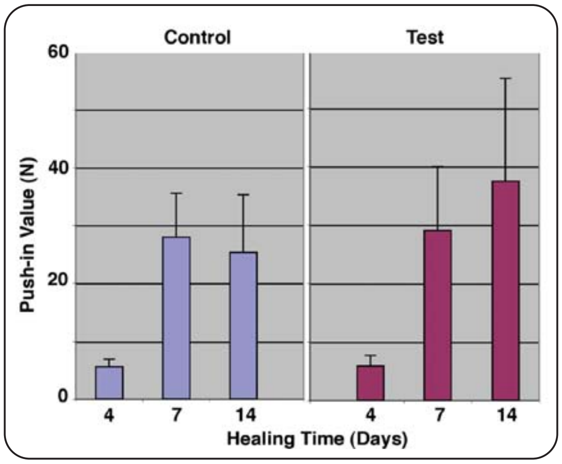


Figure 1: Implant push-in test in the rat femur model. All implants have the DAE surface. Test implant surfaces were additionally treated with DCD.

Nanometer-scale CaP Enhances Early Implant-Bone Fixation In An Animal Model

James N. Kenealy, PharmD, Bruce Berckmans, Renée M. Stach, DDS, BIOMET ***3i****, Palm Beach Gardens, Florida, USA*

European Association For Osseointegration

15th Annual Meeting (October 5–7, 2006, Zurich, Switzerland)

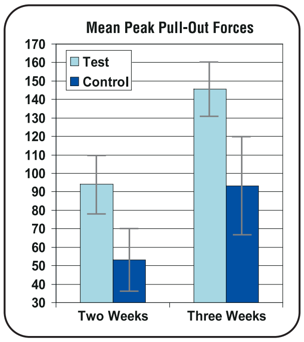
Published: *Clin Oral Implant Res.* 2006;17:cxxi.

Introduction

This study used an established rabbit tibia pull-out model to demonstrate early bone fixation of implants treated with a Discrete Crystalline Deposition™ (DCD™) of nano-scale calcium phosphate (CaP) on a dual-acid-etched (DAE) surface.

Materials And Methods

Twenty six-month-old New Zealand white rabbits were assigned to two treatment groups. Control implants were 3.3mmx4mm Ti-6Al-4V-alloy with the DAE surface. Test implants were of the same design with the addition of DCD nano-scale CaP. One of each implant was surgically placed in the right antereomedial tibia of each rabbit 15mm apart, alternating medial and distal sites between animals. Radiographs were obtained to demonstrate uniformity of implant placement. The first treatment group was sacrificed after two weeks and the second group after three weeks. Each tibia was dissected in whole, mounted and stabilized in a precision alignment apparatus. A standard Instron® machine (Model #TTMBL) was attached to apply tensile forces along the long axis of the implant. The peak pull-out force to detach the implant from bone was electronically measured and recorded.



Results And Discussion

Mean peak pull-out forces for Test implants were 94.0±33.6 N at two weeks and 145.6±19.8 N at three weeks. Mean forces for Control implants are 53.2±24.1 N at two weeks and 93.2±44.9 N at three weeks and $P<0.05$ between groups at both time points.

Conclusion

After two and three week healing periods, a significant increase in peak pull-out forces was observed for DAE implants with DCD nano-scale CaP in comparison to implants treated with DAE alone.

Discrete Calcium Phosphate Nanocrystalline Deposition Enhances Osteoconduction On Titanium-based Implant Surfaces

Vanessa C. Mendes, John E. Davies, Institute of Biomaterials and Biomedical Engineering, Faculty of Dentistry, University of Toronto, Ontario

Academy Of Osseointegration

22nd Annual Meeting (March 8-10, 2007, San Antonio, Texas)

Introduction

The surface microtopography of implants influences biological response in the peri-implant compartment by enhancing early peri-implant healing and promoting increased bone-implant contact (BIC). A recent technology has been developed, in which nano-structured calcium phosphate (CaP) crystals are deposited onto microtopographically complex titanium surfaces, adding another level of complexity to them. Our study was aimed at analyzing and comparing osteoconduction, measured as BIC, on micro and nano-textured titanium implant surfaces, using a bone ingrowth chamber model.

Materials And Methods

Custom T-shaped bone ingrowth chambers were fabricated from either commercially pure titanium (cp) or titanium alloy (Ti64). All chambers were dual-acid-etched (DAE) and half of them were modified by a Discrete Crystalline Deposition (DCD) of CaP on the surface. Four groups were generated (cp, cpDCD, Ti64, Ti64DCD) with a total of 130 implants placed into both femora of male Wistar rats for nine days. After harvesting, samples were resin embedded and back-scattered electron images of multiple planes of the implant chambers were produced. Quantitative analysis of BIC was performed on a total of 1087 micrographs.

Results And Discussion

This bone ingrowth model was effective to demonstrate the osteoconductive behavior along surfaces with differences in complexity of their microtopography. Newton-Coles numerical integration formula was used to determine bone ingrowth as a function of anatomical location of the chamber opening and the implant surface. All groups exhibited osteoconduction, but Ti64 groups showed a higher chance to grow more bone than cp, although this was not statistically significant. However, osteoconduction on both DCD groups (26.95% cpTiDCD, 29.73% Ti64DCD) was statistically significant when compared to the results of non-DCD groups (12.01% cpTi, 16.97% Ti64). The proximal opening of the chamber consistently showed significantly higher bone ingrowth (17.49% cpTi, 30.37% cpTiDCD, 23.19% Ti64, 34.88% Ti64DCD) than the distal side (8.31% cpTi, 24.46% cpTiDCD, 12.60% Ti64, 25.55% Ti64DCD). This can be explained by the direction of the predominant blood supply to the distal femur.

Conclusion

The DCD enhanced osteoconduction during early stages of peri-implant healing and that the anatomical location plays an important role in bone growth in this model.

*Discrete Calcium Phosphate Nanocrystals Render Titanium Surfaces Bone Bonding**

Vanessa C. Mendes, John E. Davies, Institute of Biomaterials and Biomedical Engineering, Faculty of Dentistry, University of Toronto, Ontario

Canadian Biomaterials Society

25th Annual Meeting (May 26–28, 2006, Calgary, Alberta, Canada)
Published: *Int J Oral Maxillofac Implant.* 2007;22:484

Introduction

The surfaces of titanium dental implants have evolved with a variety of microtextures that have been shown to accelerate early bone healing and increase implant stability.[1,2] Specifically, microtextured surfaces of either metallic or calcium phosphate (CaP) implants have been shown to improve healing by upregulation of platelet activation[3,4] and increasing fibrin retention to the implant surface; which are elements necessary to signal, and provide a transient biological matrix for, osteogenic cell migration to the implant surface that results in contact osteogenesis.[5] An additional benefit of CaP coatings – usually ≥ 50 microns thick – is that they have been shown to not only accelerate early healing but also permit bone bonding to their surfaces. The latter is a benefit unobtainable with metallic surfaces.[6] Nevertheless, CaP plasma-sprayed implants also have demonstrated clinical problems due to delamination and resultant particulate formation in vivo which have led to inflammatory responses and implant failure under loading. For this reason, particularly in the dental implant field, CaP-coated surfaces have been largely replaced by microtextured metallic surfaces.

Recently, it has been shown that it is possible to modify an already clinically successful microtextured metallic surface by the deposition of discrete nano-crystals of CaP. These deposited crystals have little effect on the micron-scale texture of the metallic surface, but do superimpose upon it a nano-scale topographical complexity. Indeed, we show elsewhere at this conference that such discrete CaP crystalline deposits (DCD™) can significantly accelerate osteoconduction. The purpose of the present study was to design an experiment to ask the question: Can such CaP/DCD render a metallic implant surface bone-bonding? To address the question, we designed custom implants to measure the strength of attachment of bone to a candidate implant surface.

Materials And Methods

Custom designed rectangular implants, 6x4x1.5mm, were fabricated from either commercially pure ("cp") or titanium alloy (Ti6Al4V or "alloy"). Each implant had a central hole down the long axis to enable suture fixation at surgery. All implants were dual-acid-etched (DAE) using either H_2SO_4/HCl or HCl/HF for cp and alloy implants respectively. Half of the cp and alloy implants were modified by the CaP/DCD (nominal crystal size 20nm; surface coverage approx. 50%; modified particulate sol-gel deposition method). Thus, a total of four groups: cp, cpDCD, alloy, and alloyDCD were generated. Forty eight implants (12 per group) were placed antero-posteriorly into the distal metaphyses of both femora of male Wistar rats for nine days. The femora of the sacrificed animals were trimmed to the width of the implant and placed in sucrose buffer. The resulting samples consisted of two cortical arches of bone attached to each implant (n=96). For each sample, nylon lines were passed through the marrow spaces between the implant and each cortical arch and the implant was secured in a vice attached to an Instron® Testing Machine (model 8501) (Figure 1). Each nylon line was then attached to the Instron Frame, displaced at a crosshead speed of 30mm/min, and the force to rupture the sample was recorded. Thus, for each implant, two force/displacement results were generated, one for each femoral arch (medial or lateral). Scanning electron microscopy (SEM) was used as a qualitative method to analyze the bone/implant interface following the detachment assay.

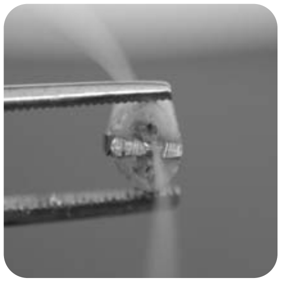


Figure 1: Preparation of sample for the tensile test.

Results And Discussion

The post-operative period was uneventful for all rats, except one, which had problems load-bearing and was excluded from the study. One sample was compromised while being positioned in the Instron Machine. Thirty two arches (16 cp, 2 cpDCD, 12 alloy and 2 alloyDCD) were insufficiently attached to the surface of their implant to withstand sample handling. These data were included in the study. The resulting 63 samples were tested as described above. DCD implant surfaces, either cp or alloy, had statistically significantly greater average values of detachment force (cp 7.08N and alloy 11.30N) than controls (cp 0.60N and alloy 1.90N) (Figure 2). Furthermore, DAE alloy surfaces exhibited statistically significant higher detachment force results than

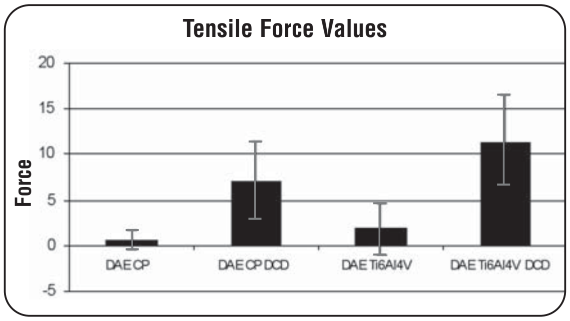


Figure 2: Tensile force values for all four groups.

*Bone Bonding™ is the interlocking of bone with the implant surface.

DAE cp. This was expected and could be explained by the enhanced microtopographical complexity of the alloy surface as compared to the cp surface.

The bone-bonding phenomenon was demonstrated in this study by visual inspection of the samples (Figure 3) and SEM. In samples with high "detachment" forces, the bone arches had fractured while the bone in contact with the implant surface was not detached. No such bone-bonding was observed in the DAE samples.

Figure 3: Bone-bonding phenomenon.

Conclusion

DAE implant surfaces can be improved and rendered bone-bonding by nano-structured CaP surface modification; and DAE alloy demonstrates greater bone attachment than DAE cp titanium.

References

1. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. ***Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs.*** *J Biomed Mater Res* 1991;25:889-902
2. Gotfredsen K, Wennerberg A, Johansson C, Skovgaard LT, Hjorting-Hansen E. ***Anchorage of TiO2-blasted, HA-coated, and machined implants: an experimental study with rabbits.*** *J Biomed Mater Res* 1995;29:1223-31
3. Park JY, Davies JE. ***Red blood cell and platelet interactions with titanium implant surfaces.*** *Clin Oral Implant Res* 2000;11:530-9
4. Kikuchi L, Park JY, Victor C, Davies JE ***Platelet interactions with calcium phosphate coated surfaces.*** *Biomaterials* 2005;26:5285
5. Davies JE. ***Understanding peri-implant endosseous healing.*** *J Dent Educ* 2003;67:932-49
6. Jarcho M, Kay JI, Gummaer RH, Drobeck HP ***Tissue, cellular and subcellular events at a bone-ceramic hydroxylapatite interface.*** *J. Bioeng* 1977;1:79

Acknowledgements: CIHR Fellowship (VCM), Grants from ORDCF and BIOMET ***3i***, Palm Beach Gardens, Florida (JED)

Discrete Calcium Phosphate Nanocrystals Enhance Osteoconduction On Titanium-based Implant Surfaces

Vanessa C. Mendes, John E. Davies, Institute Of Biomaterials And Biomedical Engineering, Faculty Of Dentistry, University Of Toronto, Ontario

Canadian Biomaterials Society

25th Annual Meeting (May 26–28, 2006, Calgary, Alberta, Canada)

Introduction

Contact osteogenesis, or the apparent growth of bone on (or along) an implant surface, is the result of both osteoconduction, which we have defined as the "recruitment and migration of osteogenic cells", and the subsequent secretory activities of these migrated cells which results in the elaborated bone matrix. The surface microtopography of implants has been demonstrated to influence biological response in the peri-implant compartment and, specifically, to enhance early peri-implant healing and promote increased bone-implant contact when compared to smooth surfaces.[1,2,3] These mechanisms have now been thoroughly documented.[4,5] We have previously described a bone-ingrowth chamber model to quantitatively measure osteoconduction, normally referred to as Bone Implant Contact (BIC).[2] However, without further refinements of the model, we have not, until now, employed this method to compare osteoconduction on metallic surfaces of essentially similar microtopography. We describe herein, the detailed design of, and refinements to, this miniature bone ingrowth chamber model; the processing techniques we have developed to derive histological data from multiple samples in parallel; the block face preparation and back-scattered electron imaging techniques we employ to derive multiple planes of examination from each multiple sample group; and the means of quantitatively analyzing the experimental results.

Our results show that etched titanium alloy is more osteoconductive than commercially pure titanium. When modified by nano-scale deposits of calcium phosphate crystals, osteoconduction on both metals is further significantly increased.

Materials And Methods

Custom designed T-shaped implants ("Tplants"), 5 x 3 x 2mm, were fabricated by BIOMET ***3i*** in Palm Beach Gardens, Florida, USA, from either commercially pure ("cp") or titanium alloy (Ti6Al4V or "alloy"). The stem of the "T" is hollow with a cavity 3x2x1mm into which bone could grow following implantation (Figure 1). The Tplants are constructed in modular parts so that their inner walls can be surface modified before assembly. On one external surface, a notch was laser-etched in an angle of 45°, to enable calculation of the height of each cross section of the chamber during sample analysis. All implants were dual-acid-etched (DAE) using either H_2SO_4/HCl or HCl/HF for cp and alloy implants respectively. Half of the cp and alloy implants were modified by the CaP/DCD (nominal crystal size 20nm; surface coverage approx. 50%; modified particulate sol-gel deposition method). Thus, a total of four groups: cp, cpDCD, alloy and alloyDCD were generated. Four groups of 25 Tplants of each experimental group (total=100), were placed antero-posteriorly into the distal metaphyses of both femora of male Wistar rats for nine days (Figure 2). After harvesting, individual samples (Figure 3) were mounted in groups of 10 on custom plate holders (Figure 4) and resin embedded for block-face microscopy (Figure 5). The block surface was repeatedly ground (automated polishing machine, EcoMet 3000 & AutoMet 200 Power Head, Buehler, IL, USA) to produce back-scattered electron images (BSEI), following platinum sputter coating, of different planes of the implant chamber (Figures 6 and 7). Quantitative analysis of BIC was performed with the software Sigma Scan Pro 4 (SPSS Inc.) on >450 micrographs. Single and multiple analysis of variance (ANOVA) were applied to bone contact measurements as a function of metal (cp vs. alloy) and modification with CaP. All statistical analysis were conducted using JMP v5.0.1 software (SAS Institute, Inc., Cary, NC). Statistical significance was considered at $p < 0.05$ and a Tukey-Kramer HSD post hoc analysis was applied to mean comparisons where indicated by an overall statistical influence.

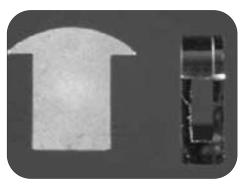


Figure 1

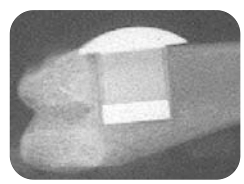


Figure 2

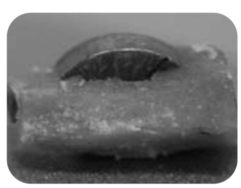


Figure 3

Figure 4

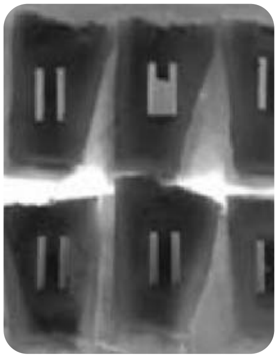


Figure 5

Figure 6

Figure 7

Results And Discussion

The post-operative period was uneventful. In a total of 100 femurs, there were 3 fractures. Those samples were excluded from the study. Our initial analysis indicated osteoconduction in all groups. Osteoconduction was identified by bone growth along the walls of the implant. Osteoconduction on both DAE-DCD groups (cpTi and Ti6Al4V) was significantly enhanced in all levels of analysis. This was statistically significant when compared to the results of DAE groups in all levels of analysis (independent measurements and aggregate measurements per layer and per implant). Interestingly, when the metals (cpTi or Ti6Al4V) were isolated, it was demonstrated that there was no statistical difference between both metals in an analysis of aggregate measurements per implant. However, statistically significant differences were verified, with respect to increased values of osteoconduction on Ti6Al4V, in an analysis of either aggregate measurements per layer or independent bone contact measurements.

This study has also shown, in a consistent manner, a variation of the amount of bone-implant contact dependent on anatomical location. The proximal side of the chamber had significantly higher bone-implant contact than the distal side. It is suggested that the direction of the vascular supply flow (from proximal to distal) in the rat femur would have been responsible for that.

Conclusion

Our results show that this bone ingrowth model can be employed to demonstrate the osteoconductive behavior along surfaces which display only minor differences in complexity of their microtopography.

References

1. Buser D, et al (1991) *J Biomed Mater Res* 25:889-902
2. Dziedzic, DM & Davies, JE. *20th Ann Meeting of the Soc. for Biomaterials.* April 1994, Boston, USA
3. Lazzarra, R.J. et al. (1999) *Int. J. Periodontics Restorative Dent.* 19:117-29
4. Davies, J.E. (2003) *J. Dent. Educ.* 67:932-949.
5. Davies JE & Hosseini, MM (2000) in: Davies J. E., *Bone Engineering.* Toronto: em squared Inc, p. 1-14

Acknowledgements: CIHR Fellowship (VCM), Grants from ORDCF and BIOMET ***3i,*** Palm Beach Gardens, Florida (JED)

Discrete Calcium Phosphate Nanocrystals Render Titanium Surfaces Bone Bonding

Vanessa C. Mendes, John E. Davies, Institute Of Biomaterials And Biomedical Engineering, Faculty Of Dentistry, University Of Toronto, Ontario

Academy Of Osseointegration

22nd Annual Meeting (March 8-10, 2007, San Antonio, Texas)

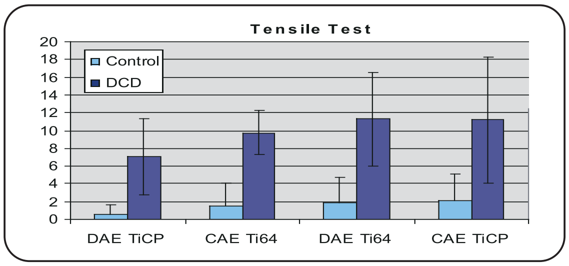
Introduction

Recently, an already clinically successful microtextured metallic surface has been modified by the Discrete Crystalline Deposition™ (DCD™) of nano-crystals of calcium phosphate (CaP). These deposited crystals have little effect on the micron-scale texture of the metallic surface, but do superimpose upon it a nano-scale topographical complexity. The purpose of the present study was to design an experiment to answer the question: Can such DCD render a metallic implant surface bone-bonding?

Materials And Methods

Custom designed rectangular implants, 1.3x2.5x4mm, were fabricated from either commercially pure (cp) or titanium alloy (Ti6Al4V or Ti64). Implants were either dual-acid-etched (DAE) or treated by a citric acid based process (CAE), and half of the implants were modified by the DCD. Eight groups were generated and implants were placed bilaterally into the femora of 48 male Wistar rats for nine days. After harvesting, the femora were trimmed to the width of the implant, resulting in two cortical arches of bone attached to each implant. The implant was attached an Instron® Testing Machine and the arches of bone were distracted at 30mm/min. The force to rupture the sample was recorded. Field emission scanning electron microscopy (FESEM) was used to analyze the bone/implant interface following detachment.

The post-operative period was uneventful except for one rat, which had problems load-bearing and was excluded from the study. Two samples were compromised while being positioned in the Instron machine. Sixty one arches (16 cpDAE, 2 cpDAE-DCD, 12 Ti64DAE, 2 Ti64DAE-DCD, 13 cpCAE, 14 Ti64CAE and 2 cpCAE-DCD) were insufficiently attached to the surface of their implant to withstand handling, and thus were included as zero. The resulting 129 arches were mechanically tested. DCD surfaces had statistically significantly greater average values of detachment force (cpDAE-DCD 7.08N, Ti96DAE-DCD 11.30N, cpCAE-DCD 11.19, Ti64CAE-DCD 9.74) than non-DCD (cpDAE 0.60N, Ti64DAE 1.90N, cpCAE 1.49, Ti64CAE 9.74). The bone-bonding phenomenon was visually evident when the cortical arches were fractured and the bone/implant interface remained intact. Globular accretions similar to those of cement lines were observed microscopically, by FESEM, interdigitating with the surface of the implant. We conclude that titanium implant surfaces can be improved and rendered bone-bonding by nano-structured CaP surface modification.



Discrete Deposition Of Hydroxyapatite Nanoparticles On A Titanium Implant With Predisposing Substrate Microtopography Accelerated Osseointegration

Ichiro Nishimura, Frank Butz, Takahiro Ogawa, Audrey Lin and Chiachien Jake Wang,
UCLA School of Dentistry, The Weintraub Center for Reconstructive Biotechnology and Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, Los Angeles, CA, USA, Yuhong Huang, Chemat Technology, Incorporated, Northridge, CA, USA

We report here a new versatile method to deposit discrete hydroxyapatite (HA) nanoparticles on a titanium (Ti) implant with predisposing substrate microtopography, which exhibited an unexpectedly robust biological effect. Commercially pure Ti substrates were treated with 3-aminopropyltriethoxysilane, on which HA nanoparticles (20 nm) were deposited and chemically bonded to TiO_2. The HA deposition rate was linearly related to the treatment time and HA nanoparticles were deposited on up to 50% of the substrate surface. As a result, the discrete deposition of HA nanoparticles generated novel 20–40nm nanotopography on the Ti substrate with microtopography that was smooth (turned) or roughened by double acid etching (DAE). The experimental implants with or without HA nanoparticles were surgically placed in rat femur and an implant push-in test was performed after two weeks of healing. The deposition of HA nanoparticles on the DAE surface increased the mechanical withstanding load by 129% and 782% as compared to the control DAE and turned implants, respectively. Micro-computed tomography-based 3D bone morphometry revealed equivalent bone volumes around the DAE implant with or without HA nanoparticles. These data suggest that the discrete deposition of HA nanoparticles accelerates the early osseointegration process, likely through increased shear bonding strengths.

Continued on next page

Discrete Deposition Of Hydroxyapatite Nanoparticles On A Titanium Implant With Predisposing Substrate Microtopography Accelerated Osseointegration

Continued from previous page

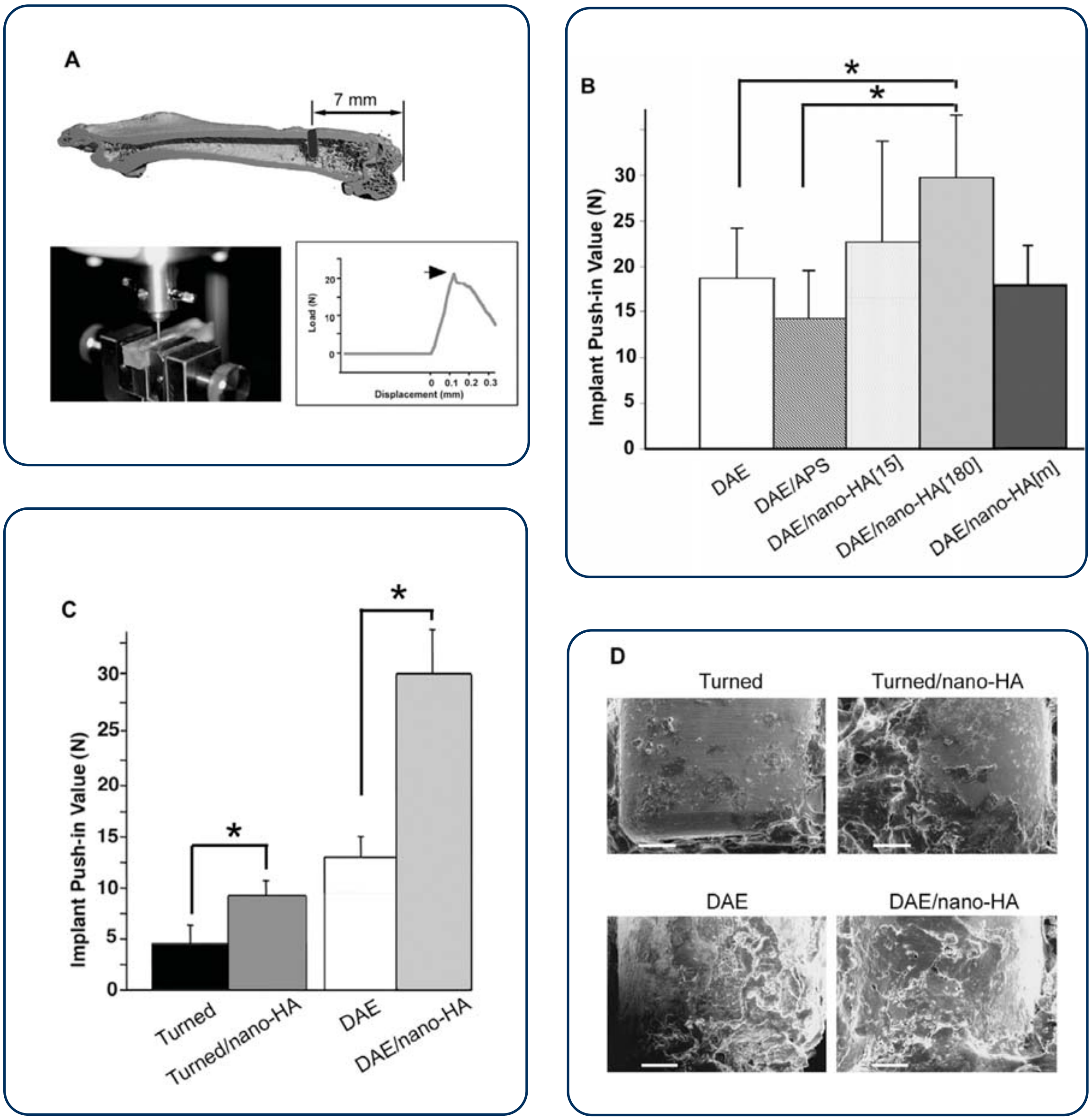


Figure 1: Rat implant push-in tests. (A) Micro-CT image of rat femur with a miniature Ti implant placed at 7 mm from the proximal end (top panel). After two weeks of healing time, the femur-implant specimen was embedded in a resin block and subjected to electromechanical loading (bottom-left panel). The maximal load prior to the implant–bone shear was used as the implant push-in value (arrow in bottom-right panel). (B) Implant push-in values measured at day 14. Test implants were DAE–Ti implants (DAE; n = 5), DAE–Ti implants with the first APS layer (DAE/APS; n = 3), APS-treated DAE–Ti implants immersed in nano-HA colloidal solution for 15 min (DAE/nano-HA[15]; n = 3) or 180 min (DAE/nano-HA[180]; n = 5), and DAE–Ti implants coated with multilayers of HA nanoparticles (DAE/nano-HA[m]; n = 5). Error bars indicate 1SD. = $p < 0.05$ (C) implant push-in values of Ti implants of turned Ti implants with smooth microtopography (turned; n = 5), turned implants with nano-HA coating (turned/nano-HA; n = 5), DAE-treated Ti implants with rough microtopography (DAE; n = 5), and DAE implants with nano-HA coating (DAE/nano-HA; n = 5) measured at day 14 of implantation. Error bars indicate 1SD (one standard deviation). = $p < 0.05$ (D) SEM analyses of the implant surface after push-in testing. Bar = 200 μm.

Discrete Deposition Of Calcium Phosphate Nanocrystals Promotes Bone-Bonding On Titanium Surfaces

Vanessa C. Mendes, Rahim Moineddin, John E. Davies, Institute Of Biomaterials And Biomedical Engineering, Faculty Of Dentistry And Department Of Family & Community Medicine, University Of Toronto, Canada

Society For Biomaterials

32nd Annual Meeting (April 18-21, 2007, Chicago, IL, USA)

Statement of Purpose

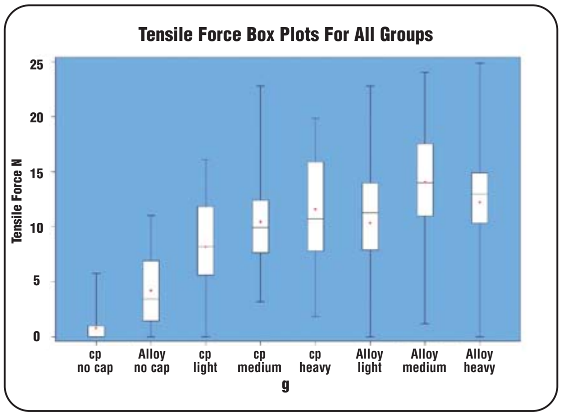
The surfaces of titanium dental implants have evolved with a variety of microtextures that have been shown to accelerate early bone healing and increase implant stability.[1,2] Recently, it has been found that it is possible to modify an already clinically successful microtextured metallic surface by the Discrete Crystalline Deposition™ (DCD™) of nano-crystals of calcium phosphate (CAP). These deposited crystals have little effect on the micron-scale texture of the metallic surface, but do superimpose upon it a nano-scale topographical complexity. The purpose of the present study was to design an experiment to ask the questions: (a) Can such nanofeatures render a metallic implant surface bone-bonding? and (b) Does the amount of CAP deposition influence the degree of bone-bonding? To address these questions, we designed custom implants to measure the attachment of bone to candidate implant surfaces.

Methods

Custom designed rectangular implants, 1.3x 2.5x4mm, were fabricated from either commercially pure ("cp") or titanium alloy (Ti6Al4V or "alloy"). All implants were dual acid etched (DAE) [H_2SO_4/HCl or HCl/HF for cp and alloy respectively]. A total of 8 groups (2 non-DCD and 6 DCD) were generated and 13 implants were fabricated for each group. Non-DCD groups consisted of DAE cp and alloy without any CAP deposition. DCD groups comprised the original DAE cp and alloy implants modified by the CAP nanocrystals (nominal crystal size 20-80nm). Implant surfaces had different CAP crystals deposition and the groups (either cp or alloy) were: light (25% surface coverage), medium (60% surface coverage) and heavy (90% coverage). One implant of each group was submitted to Field Emission Scanning Electron Microscopy (FESEM) and 96 implants (12 per group) were placed into the distal metaphyses of both femora of male Wistar rats for 9 days. The femora of the sacrificed animals were trimmed to the width of the implant and the resulting samples consisted of 2 cortical arches of bone attached to each implant. For each arch, in turn, a nylon line was passed under the cortical, through the marrow space. The implant was attached to a sample holder and secured to an Instron™ testing machine and the arch was distracted at crosshead speed of 30mm/min. The force to rupture the sample was recorded and analyzed. FESEM was used as a qualitative method to analyze the bone/implant interface following the detachment assay.

Results/Discussion

The post-operative period was uneventful for all rats and none was excluded from the study. Two samples were compromised while being positioned in the Instron™ machine. Twenty-three arches (15 cp DAE, 1 cp-DCD light, 4 alloy DAE, 2 alloy-DCD light and 1 alloy-DCD heavy) were insufficiently attached to the surface of their implant to withstand sample handling. These data were included in the study. The resulting 169 arches were tested as described above. Distributions of tensile forces were not skewed except for group cp DAE which has 60% zero values for tensile forces. There were no significant differences between left and right leg, ($p=0.2227$) and between arches ($p=0.0512$). When comparing the cp and alloy groups, the mean for 'alloy' was higher than the mean of tensile forces for 'cp' ($p<0.0001$). The comparisons of 8 groups showed that the two non-DCD groups were the same ($p=0.3925$). The average of tensile forces for both non-DCD groups was statistically significant lower than for DCD groups ($p<0.05$). The comparison among DCD groups showed that the means of tensile forces for all groups were statistically the same except for group cp-DCD light ($p=0.0384$). The bone-bonding phenomenon was demonstrated by visual inspection of the samples and FESEM. In samples with high "detachment" forces, the cortical bone arches had fractured while the bone in contact with the implant surface was not detached. Group cp-DCD light had the lowest amount of bone remaining on the surface of the implant. This finding corroborates the statistically significantly lower tensile force values for this group, which could be explained by a more fragile and less resistant bone-implant attachment at the interface.



Conclusions

The DCD surface treatment renders the implant surface bone-bonding, which was demonstrated by the fracture of the bone above the bone-implant interface. Alloy groups were able to promote a better bone-implant attachment than cp groups. The amount of CAP deposition influences the detachment force, however, the failure force reaches a plateau beyond which increasing the deposited CAP has no effect.

References

1. Buser D et al. J Biomed Mater Res. 1991, 25:889-902

2. Gotfredsen K et al. J Biomed Mater Res. 1995; 29:1223.

Human Histological Studies

Randomized, Controlled Histologic And Histomorphometric Evaluation Of Implants With Nanometer-Scale Calcium Phosphate Added To The Dual Acid-Etched Surface In The Human Posterior Maxilla*

Giovanna Orsini, Maurizio Piattelli, Antonio Scarano, Giovanna Petrone, Adriano Piattelli, Sergio Caputi,
Operative Unit Of Stomatology And Oral Science Of Center For Excellence On Aging - "G. d'Annunzio" University Foundation And Department Of Stomatology And Oral Science, University Of Chieti-Pescara, Chieti, Italy
*James Kenealy, BIOMET **3i**, Palm Beach Gardens, FL*

Background
Placement of dental implants in the posterior maxilla has been associated with higher rates of failure that are due, in part, to the poor bone quality of this region. The purpose of the present study was the histologic and histomorphometric evaluation of the bone around a new implant surface treatment created by a deposition of nanometer-sized calcium phosphate particles added to the dual acid-etched surface.

Methods
One custom-made 2 x 10-mm site evaluation implant (SEI) with this novel treatment surface (test) and one SEI with the dual acid-etched surface without treatment (control) were placed in the posterior maxilla of 15 patients. All SEIs were retrieved after two months and evaluated under confocal laser scanning microscopy (CLSM) and by light microscopy for histomorphometric analysis of the bone-implant contact (BIC).

Results
Histologic observations in control SEIs showed formation of new bone around the implant surface; however, it was not always in direct contact with the entire perimeter of the threads. The mean BIC was 19% ± 14.2%. Test SEIs showed peri-implant bone tightly contacting the implant surface and better adapted to the threads. Three-dimensional reconstruction of sections obtained using CLSM showed the intimacy of the contact between bone and test SEI surface through the entire thickness of the specimens. The mean BIC was 32.2% ± 18.5%.

Conclusions
After 2 months of healing, comparison of the BIC values showed a statistically significant greater mean BIC for test SEIs than for controls. The clinical implications of these results included shortening of implant healing period and earlier loading protocols.

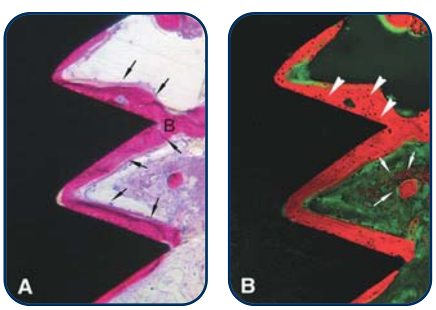


Figure 1: ***A)*** Histologic view of test SEI. The bone (B) is adapted well to the entire perimeter of the implant threads (arrows). (Acid fuchsin and toluidine blue; original magnification x10.) ***B)*** The CLSM image allows improved identification of osteocytes (arrowheads) in the peri-implant bone and helps in the detection of some newly formed bone in the interthread region (arrows) (original magnification x10).

Table 1: Summary Of BIC For SEIs

Patient Number	Control SEIs (BIC%)	Test SEIs (BIC%)
1	4.3	45.1
2	54.1	0
3	40.0	52.0
4	24.0	65.1
5	3.0	23.0
6	15.3	22.4
7	30.3	15.0
8	19.6	47.7
9	8.1	53.1
10	7.2	47.0
11 (Case 1)	9.8	19.0
11 (Case 2)	19.8	13.5
12	53.1	22.0
13	13.0	7.1
14	18.1	19.0
15	0	16.3
Summary		
N	16	16
Mean	20.0	29.2
SD	16.71	19.31
Minimum	0	0
Maximum	54.1	65.1
Excluding Patients With "0" Values		
N	14	14
Mean	19.0	32.2
SD	14.18	18.49
Minimum	3.0	7.1
Maximum	53.1	65.1

Table 2: Statistical Analysis Of Treatment Difference In BIC Percentage

Summary	Excluding "0" Values
N	14
Mean Difference	13.26
SD of Difference	23.57
Minimum Difference	-31.05
Maximum Difference	44.98
P value (two-sided paired t test)	0.0554
P value (one-sided paired t test)	0.0277
P value (two-sided Wilcoxon signed-rank)	0.0580
P value (one-sided Wilcoxon signed-rank)	0.0290

***Full results published:** *J Periodontal* 2007;78:209-218

*Influence Of A Nanometer-Scale Surface Enhancement On De Novo Bone Formation On Titanium Implants: A Histomorphometric Study In Human Maxillae**

Ronnie J. Goené, DDS, Clinical Professor, Department Of Oral And Maxillofacial Surgery/Pathology, Academic Centre For Dentistry Amsterdam, VU Medical Center, Amsterdam, The Netherlands
Tiziano Testori, MD, DDS, Head, Section of Implantology, Department Of Odontology, University Of Milan, Italy; Private Practice, Como, Italy
Paolo Trisi, DDS, PhD, Scientific Director, Biomaterials Clinical Research Association, Pescara, Italy

In this prospective randomized controlled clinical study, small titanium implants were placed in the posterior maxillae for the purpose of assessing the rate and extent of new bone development. Nine pairs of site evaluation implants were placed in posterior areas of the maxillae and retrieved with trephine drills after 4 or 8 weeks of unloaded healing. The amount of bone in linear contact (%) with the implant surface was used to determine the osteoconductive potential of the implant surface. Implant surfaces were dual acid etched (n = 9) (controls) or dual acid etched and further conditioned with nanometer-scale crystals of calcium phosphate (n = 9) (test implants), and the surfaces were compared. The implants and surrounding tissues were processed for histologic analysis. The mean bone-to-implant contact value for the test surface was significantly increased over that of the control implants at both time intervals (P < .01). For the implants/patients included in this study, the addition of a nanometer-scale calcium phosphate treatment to a dual acid–etched implant surface appeared to increase the extent of bone development after 4 and 8 weeks of healing.

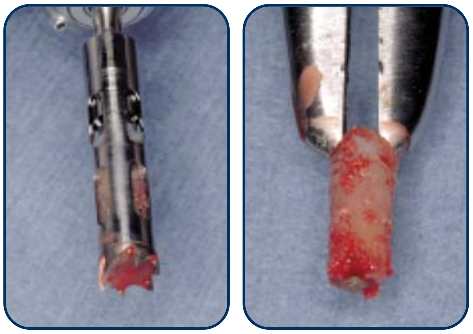


Figure 1: (left) Trephine and core samples, along with the 4.25-mm trephine drills used to recover the implants and tissue. (right) The extricated core sample shows the SEI in the middle of an intact bone core.

Table 1: Histomorphometric outcomes of SEI analyses

		%BIC		% BV	
Pair	**Weeks**	**Nanotite™**	**OSSEOTITE®**	**Nanotite**	**OSSEOTITE**
1	4	47.1	13.7	26.1	36.8
2	4	50.2	20.1	17.6	21.7
3	4	36.1	11.1	31.9	22.6
4	8	44.0	35.9	28.3	28.9
5	8	30.1	18.5	31.9	17.5
6	8	54.8	22.0	41.9	16.9
7	8	39.3	25.9	15.6	24.6
8	8	84.0	0.0	30.2	0.0
9	12	19.7	7.3	34.5	24.8
Overall		45.0 ± 18.1	17.2 ± 10.6	28.7 ± 8.2	21.5 ± 10.1
4-week mean		44.5 ± 7.4	15.5 ± 4.6	25.2 ± 7.2	27.0 ± 8.5
8+-week mean*		45.3 ± 22.4	18.3 ± 12.9	44.5 ± 7.4	18.8 ± 10.3

***Includes data from the SEIs retrieved at 12 weeks.**

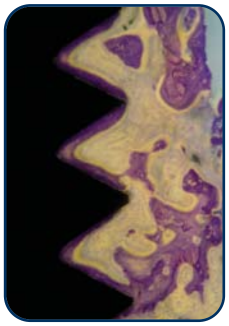


Figure 2: Nanotite surface at 8 weeks. A layer of bone about 100 µm thick covers the implant surface with osteoid and osteoblasts, suggesting active bone healing (toluidine blue; x50).

***Full results published:** *Int J Periodontics Restorative Dent* 2007;27:211–219

Overview: Studies In Progress

The following clinical protocols are either ongoing or about to be launched. A brief description of the project and its goals and objectives are described:

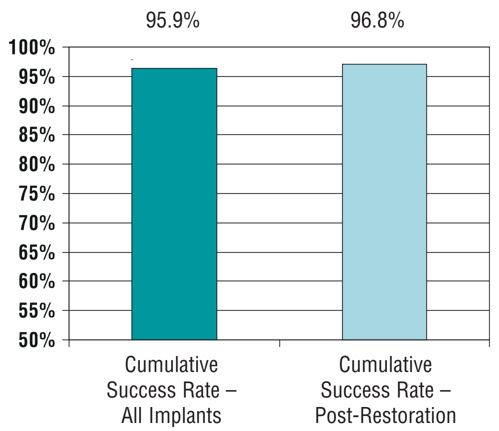
NanoTite™ Clinical Research Activity - translating preclinical results to clinical benefits

Preclinical and bench-top studies offer opportunities for determining differences between various prototype designs. The preclinical studies from UCLA and The University of Toronto go a long way to confirm that the addition of the Discrete Crystalline Deposition™ Treatment imparts a substantive improvement as compared to the standard OSSEOTITE® Surface.

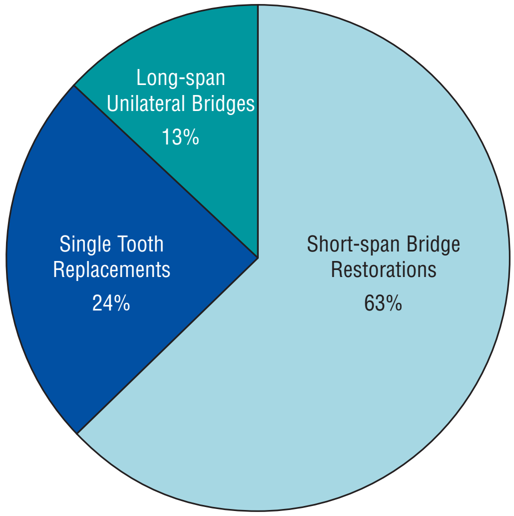
But will this matter in clinical cases? What exactly can we look to offer in terms of benefits to the clinician and patient? These and other studies to follow will provide evidenced-based data allowing clinicians to understand how they can derive additional benefit from BIOMET ***3i*** NanoTite Implants. What follows are the various ongoing research activities being conducted on the NanoTite Implant.

Immediate Loading – Study 2601 is a prospective observational study of NanoTite PREVAIL® Implants used for immediate loading of single tooth restorations and unilateral bridge cases. The goal of this project is to recruit, treat and monitor the progress of a large group of patients having a provisional restoration attached within 48 hours. Fifteen study centers located in North America, Europe and Australia are participating in this five year clinical trial. The enrollment phase is now complete and the interim results are providing the reassurance that was hoped for in bringing the benefits of immediate loading with the same overall implant success rates observed for early- and delayed-loading cases. A manuscript of the initial results is being prepared for publication.

As of August, 2007, 190 patients having 220 cases supported by 343 implants are being monitored with more than 60 patients having exceeded 12 months of on-going evaluation. Case distribution is: short-span bridge restorations (63%), single tooth replacements (24%) and long-span unilateral bridges of five units or greater (13%). Fourteen implants have been reported as failures resulting in a 95.9% overall success rate. As three of the fourteen were the result of an immediate post-surgical infection in one patient, the cumulative success rate post-restoration is 96.8%.



Cumulative Success Rates



NanoTite PREVAIL Implant Cases Under Evaluation

Overview: Studies In Progress

Human Clinical Studies

Fully Edentulous Maxilla – Study 2607 is a prospective, randomized-controlled study of fully edentulous maxilla patients where cases will be randomly assigned to receive either the NanoTite™ or OSSEOTITE® Surface Implant. The study will assess the integration success and duration of failure-free function of all implants to determine if the NanoTite Surface enhancement can improve performance in fully edentulous maxillary cases.

Placement In Augmented Bone – Study 2604 is a prospective, randomized-controlled comparison of NanoTite PREVAIL® Implants used in sinus lift augmentation sites. The goal of this multicenter study is to determine if the NanoTite Surfaced Implants placed simultaneously with the sinus augmentation graft material have the same success outcomes as do NanoTite Implants placed into healed sinus augmentation grafts. When this is established, there will be the opportunity to avoid four months of delay in functionalizing such sinus augmentation cases.

Immediate Replacement In Extraction Sites – Study 2605 is a prospective, randomized-controlled study of patients with multiple tooth extractions where at least two sites will be randomly assigned to receive either the NanoTite or OSSEOTITE Surface PREVAIL Implant. The study will assess the integration success and duration of failure-free function of all implants to determine if the NanoTite Surface enhancement can improve performance in these more challenging cases.

Sinus Augmentation Avoidance Trials – Studies 2611 and 2612 will utilize a 7mm length NanoTite Surface Implant (actual length – other "short implants are actually 7mm or longer) to be used in thin maxillary cases where a sinus augmentation (and standard length implants) would be utilized. In addition to assessing integration and duration of failure-free performance of these short implants, a specific effort will be made to quantify the amount of resources (clinician and patient time and discomfort, surgical costs, materials costs) that can be preserved by obviating the need for an augmentation surgery.

Implant Stability – Study 2610 is a randomized, double-blind evaluation of the impact of a surface modification on the Implant Stability Quotient during the initial implant healing period. This prospective, double blind, randomized-controlled clinical study will evaluate changes in the implant stability quotient (ISQ) that take place within the first eight weeks following implant placement in the posterior mandible and maxilla to determine if a difference in the ISQ measurements are detected between OSSEOTITE and NanoTite Implants.

Animal Pre-Clinical Studies

Soft And Hard Tissue Analysis Of NanoTite And OSSEOTITE Implant Surfaces –
The Sahlgrenska Academy at Göteborg University
Eight implant sites in each of six canine animals are randomly assigned to receive either NanoTite or OSSEOTITE Transgingival Implants where the surfaces extend from the apex to the coronal seating platform. After two and four weeks of healing, the interfacial tissues are examined by ground section and by a fractionation method that allows observation of microcellular structures.

Soft And Hard Tissue Analysis Of NanoTite And OSSEOTITE Implant Surfaces –
A collaborative research effort of the Universities of Madrid, Sienna and Göteborg
Four implant sites in each of six canine animals are randomly assigned to receive either NanoTite or OSSEOTITE Implants that are placed in a single-stage manner with corresponding NanoTite and OSSEOTITE Healing abutments. After two and four weeks of healing, the interfacial tissues are examined by ground section and by a fractionation method that allows observation of microcellular structures.

Authors:

Operative Unit of Stomatology and Oral Science of Ce.S.I.
"G. d'Annunzio" University Foundation and Department of Stomatology and Oral Science, University of Chieti-Pescara, Italy

Giovanna Orsini, DDS, PhD
Research Fellow

Maurizio Piattelli, MD, DDS
Professor of Oral Surgery

Antonio Scarano, DDS, MD
Researcher

Giovanna Petrone, DD, PhD
Researcher

Adriano Piattelli, MD, DDS
Professor of Oral Pathology and Medicine

Sergio Caputi, MD, DDS
Professor of Prosthodontics

University of Toronto
Institute of Biomaterials & Biomedical Engineering, Toronto, Canada

Vanessa C. Mendes, DDS, PhD
Postdoctoral Research Fellow

John E. Davies, BDS, PhD, DSc
Professor

Chunpo Pan
Undergraduate Student

Jason Chau, MSc
Graduate

Craig A. Simmons
Professor

Rahim Moineddin, PhD
Assistant Professor

Padina Pezeshki, BSc
Graduate Student

Stanley Lugowski, PhD
Research Scientist

Northwestern University
Northwestern University Institute of Nanotechnology, Evanston, Illinois

Gajendra Shekhawat, PhD
Research Assistant Professor

University of Milan
Section of Implantology, Department of Odontology, Milan, Italy

Tiziano Testori, MD, DDS
Department Head

UCLA School of Dentistry
The Weintraub Center for Reconstructive Biotechnology and Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry

Ichiro Nishimura, DDS, DMSc, DMD
Professor

Frank Butz, DDS, PhD
Research Associate, Senior Lecturer, Freiburg University

Takahiro Ogawa, DDS, PhD
Associate Professor

Audrey Lin, PhD
Research Associate

Chiachien Jake Wang, PhD
Research Associate, Postdoctoral Research Fellow, University of Connecticut

James Kelly, DDS
Prosthodontics Resident, Maxillofacial Prosthetic Fellow, MD Anderson Cancer Center

Academic Centre for Dentistry
Department of Oral and Maxillofacial Surgery/Pathology, Amsterdam, VU Medical Center, Amsterdam, The Netherlands

Ronnie J. Goené, DDS
Department Head

BIOMET *3i*
Palm Beach Gardens, Florida, USA

Bruce Berckmans
Director, Research and Development

James N. Kenealy, PharmD
Director, Global Clinical Research

Ross Towse
Director of Technology Development

Prabhu Gubbi, PhD
Senior Materials Scientist, Technology Development

Zach Suttin, MSME
Project Manager, Product Development

Alexis Goolik, MSE
Engineer, Product Development

Renée M. Stach, DDS
Medical Writer, Clinical Research

Chemat Technology, Incorporated
Northridge, California, USA

Yuhong Huang, PhD
Manager, Research and Development Division

Biomaterials Clinical Research Association
Pescara, Italy

Paolo Trisi, DDS, PhD
Scientific Director

NOTES

NOTES

Global Headquarters
4555 Riverside Drive
Palm Beach Gardens, FL 33410
1-800-342-5454
Outside The U.S.: +1-561-776-6700
Fax: +1-561-776-1272
www.biomet3i.com

Sign Up For BIOMET 3i's Electronic Newsletter "BIOMET 3innovations." Simply Go Online To www.biomet3i.com/signup

ART990
REV B 10/07

SUBSIDIARIES

AUSTRALIA
Phone: +61-2-9855-4444
Fax: +61-2-9888-9900

BELGIUM
Phone: +32-2-5410290
Fax: +32-2-5410291

BRAZIL
Phone: +55-11-5081-4405
Fax: +55-11-5081-7484

CANADA
Phone: +514-956-9843
Fax: +514-956-9844

FRANCE
Phone: +33-1-41054343
Fax: +33-1-41054340

GERMANY
Phone: +49-721-255177-10
Fax: +49-721-255177-73

IRELAND
Phone: +35-31-477-3925
Fax: +35-31-402-9590

MEXICO
Phone: +52-55-5679-1619
Fax: +52-55-5684-8098

THE NETHERLANDS
Phone: +31-(0)78-629-2800
Fax: +31-(0)78-629-2801

NEW ZEALAND
Phone: +64-508-122-221
Fax: +64-508-133-331

NORDIC REGION
Phone: +46-40-17-6090
Fax: +46-40-17-6099

PORTUGAL
Phone: +351-21-000-1645
Fax: +351-21-000-1675

SPAIN
Phone: +34-93-470-59-50
Fax: +34-93-372-11-25

SWITZERLAND
Phone: +41-44-380-46-46
Fax: +41-44-383-46 55

U.K.
Phone: +44-1628-829314
Fax: +44-1628-820182

DISTRIBUTORS

ARGENTINA
Dentalmax, SA
Phone: +541-1482-71001
Fax: +541-1482-67373

AUSTRIA
Wieladent
Phone: +43-7672-93901
Fax: +43-7672-93903

CHILE
Cybel, SA
Phone: +56-2-2321883
Fax: +56-2-2330176

CHINA
Atek Inc.
Phone: +86-21-6329-1265
Fax: +86-21-6329-1620

COLOMBIA
3i Colombia
Phone: +571-612-9362
Fax: +571-620-6412

COSTA RICA
Implantec S.A.
Phone: +506-2-256411
Fax: +506-2-247620

EL SALVADOR
Dentimerc SA de CV
Phone: +503-263-6350
Fax: +503-263-6676

GREECE
Kostas Kornisorlis and Co.
Phone: +30-2310-501-651
Fax: +30-2310-522-417

HONG KONG
Ositek Inc., Ltd.
Phone: +852-8121-6601
Fax: +852-3747-3754

ISRAEL
H.A. Systems
Phone: +972-3-6138777
Fax: +972-3-6138778

ITALY
Biomax, srl.
Phone: +39-0444-913410
Fax: +39-0444-913695

JAPAN
Implant Innovations Japan
Phone: +81-66-868-3012
Fax: +81-66-868-2444

KOREA
Jungsan Biomed Corp.
Phone: +82-2-516-1808
Fax: +82-2-514-9434

LEBANON
3i MENA s.a.l.
Middle East And North Africa
Phone: +961-1-694000
Fax: +961-1-694222

PARAGUAY
Andres H. Arce y Cia SRL
Phone: +595-21-208185
Fax: +595-21-496291

POLAND
Dental Depot
Phone: +48-71-341-3091
Fax: +48-71-343-6560

RUSSIA
Com-Dental
Phone: +7-495-797-6686
Fax: +7-499-242-9567

SINGAPORE
Asia Implant Support & Services
Phone: +65-6223-2229
Fax: +65-6220-3538

SOUTH AFRICA
Selective Surgical CC
Phone: +27-11-991-7007
Fax: +27-11-672-1391

TAIWAN
Kuo Hwa Dental Suppliers Co., Ltd.
Phone: +886-2-2226-1770
Fax: +886-2-2226-8747

THAILAND
3i (Thailand) Co., LTD.
Phone: +662-252-6685
Fax: +662-252-6686

UKRAINE
Com-Dental
Phone: +38-067-7007667
Fax: +38-044-5017117

URUGUAY
Pro3implant S.R.L.
Phone: +598-2-4034163
Fax: +598-2-4034163