The effect of a novel implant surface (Si with microblasted) on osseointegration

Project Rationale:

Hypothesis and Specific Aims:

The current standard of care for replacement of missing teeth and craniofacial defects is dental implants. Substantial time has to be allowed for implant integration with bone tissue (bone healing) prior to its restoration with a prosthesis. By changing the implant surface characteristics, bone healing has been shown to take place faster. However, little is known regarding gene expression during the healing phase of implants with different surfaces. The hypothesis for this project is that different novel implant surfaces result in different gene expression patterns during initial bone-implant integration. This hypothesis will be investigated by testing the gene expression around two different types (microblasted [MB] and Si-doped microblasted [SMB]) of novel implant surfaces. The objective of this project is to determine the gene expression pattern that results in the faster integration of endosseous implants.

Aim 1. To determine various genes expression through real-time PCR after implants of two distinct surfaces are placed in a rat femur model

Aim 2. To evaluate the interaction between bone and the different implant surfaces by histological and biomechanical testing (nanoindentation)

Significance and background:

While high survival rates have been reported for endosseous devices, current research has emphasized toward implant design modification at various length scales (i.e. macro,[1] micro,[2] and nanometric[3]) in hope to improve the early host implant tissue response. Such potential improvement in healing time may result in reduction in treatment time frames through prosthetic restorations that could be placed in occlusal function at early implantation times.[4] Among implant design modifications attempting to improve on the host-to-implant response, implant surface modifications have been the most investigated.[5,6] The rationale for surface modification lies upon the fact that it is the first part of the implant to interact with biofluids, potentially altering the cascade of events that leads to bone healing and intimate apposition with the device.[7]

From a temporal standpoint, both topographic and chemistry surface modifications have drawn attention, as both have been showing promising results in *in vitro* and *in vivo* models.[8,9] Relative to their moderately rough predecessors, improvements have been achieved by alterations in surface wettability,[10] impregnation of Ca and P onto the titanium oxide layer,[11] deposition of discrete bioactive ceramics,[12] and through minor incorporation of other chemical elements.[13] The effect of variations in surface roughness in the micrometer scale, nanometer length scale roughness, chemical impregnation with a variety of elements (such as Si,[14] Zn,[15] and Mg,[16] which are ions known to enhance bone response to biomaterials) has not been accessed to date. In addition, several *in vivo* studies have demonstrated that gene expression can be influenced by titanium surface topography.[17] A microrough implant evoked an accelerated gene expression of the bone matrix molecules osteopontin and osteonectin, along with an up-regulation of bone sialoprotein, collagen III and integrins during the first week of osseointegration.[18]

Thus, the objective of proposed study is to the effect of surface elemental chemistry (low level addition of Si with microblasted surface) on gene expression patterns during initial bone-implant integration in a rat femur model. In our collaborator, Paulo Coelho's laboratory at New York University, the surface characterization was evaluated by field-emission scanning electron microscopy (FESEM) to observe both surface topography, optical interferometry (IFM) to determine the roughness parameters, and X-ray photoelectron spectroscopy (XPS) to assess the surface-specific chemicals.[19] The surface texture observed for both surfaces at intermediate- and high-magnification levels (Fig. 1), as well as the IFM reconstruction (Fig. 2) revealed similar morphology between groups. For both micrometer and nanometer length scale IFM measurements, comparable levels were observed for both Sa and Sq values (Table 1).

Successful completion of these studies may help to understand molecular mechanisms of tissue response to novel implant surfaces, and will further provide information to aid in delineating the molecular roles of novel implant surfaces in bone regeneration. This could lead to a shorter, more predictable, and affordable treatment for partially and fully edentulous patients.

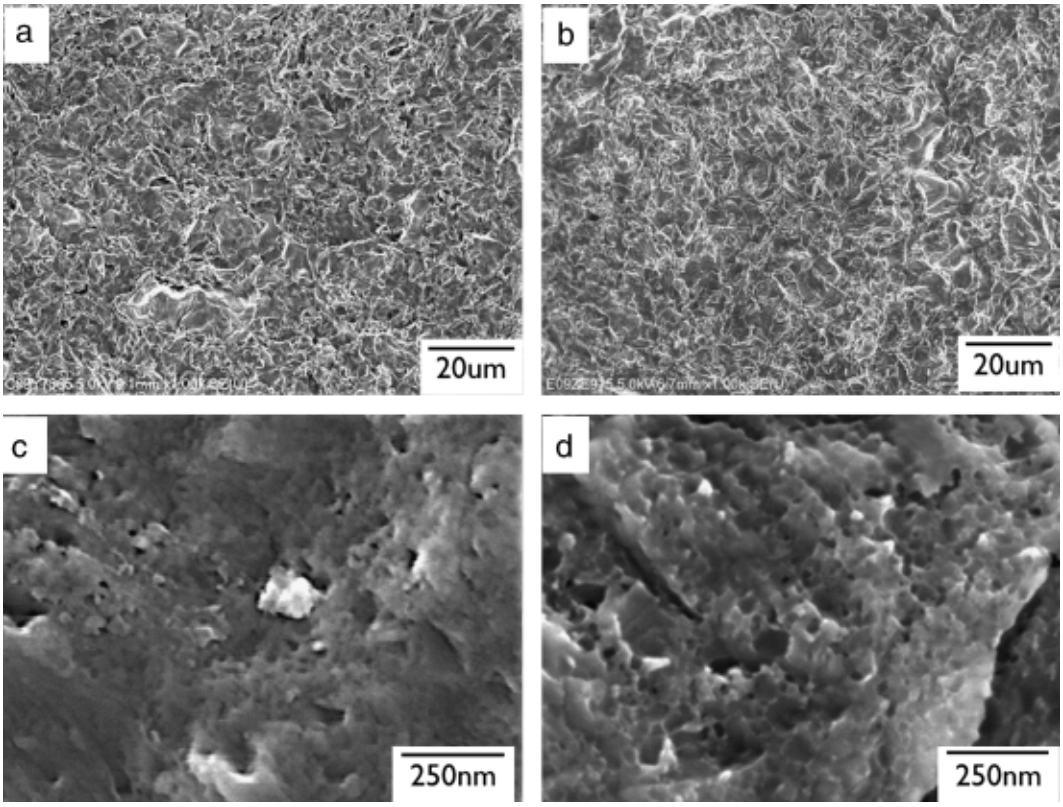

Fig. 1. FESEM of (a and c) CaP and Si Boost (b and d) presented similar roughness morphology at both low and high magnifications.

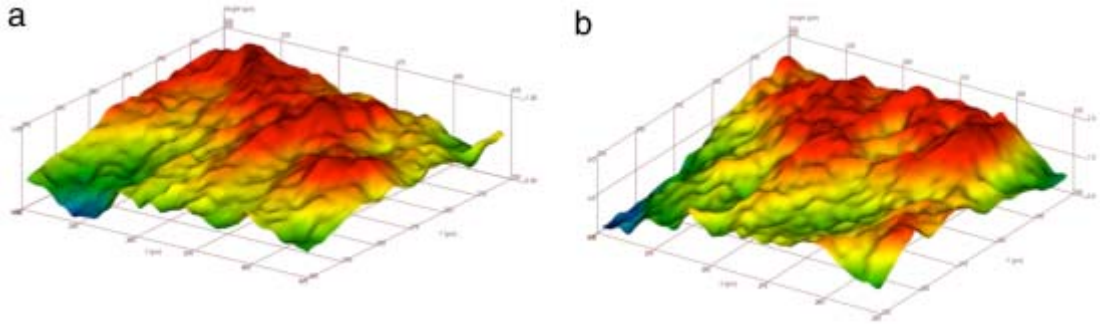

Fig. 2. Representative IFM reconstruction (filter size of 100 mm × 100 mm) of the (a) CaP and (b) Si Boost surfaces presented similar patterns.

Table 1. **Surface roughness parameters statistics summary (mean ± 95% CI) at the micrometer and nanometer level length scales**

Roughness profile length scale	S_a Si boost	S_a CaP	S_q Si boost	S_q CaP
Micrometer level roughness				
Mean	0.63	0.702	0.79	0.84
95% CI	+ 0.133	+ 0.143	+ 0.17	+ 0.198
Nanometer level roughness				
Mean	0.048	0.046	0.056	0.057
95% CI	+ 0.019	+ 0.018	+ 0.022	+ 0.022

Statistical *P* values for the micrometer level length scale S_a and S_q were >0.43, and >0.75, respectively. Statistical *P* values for the nanometer level length scale S_a and S_q were >0.9 and >0.97, respectively.

Project Protocol:

The hypothesis for this project is that different novel implant surfaces result in different gene expression patterns during initial bone-implant integration. Two different types (microblasted [MB] and Si-doped microblasted [SMB]) of novel implant surfaces will be tested to determine the gene expression pattern that results in the faster integration of endosseous implants. The applicability of these novel implants will be investigated by a rat femur model. These implants will placed in the rat femurs and then the effects of the implant surfaces on gene expression and osseointegration will be evaluated by real-time PCR, nanoindentation test, and histomorphological analysis.

Implant

Two types of surface implants, microblasted (MB), and Si-doped microblasted (SMB) (1 mm by 2 mm both from Intra-Lock) will be analyzed.

Implant Surgery

Investigating two implant surfaces (MB and SMB), a rat femur model at 8-10 weeks old will be utilized. For implant placement, and incision will be made and a surgical drill used to create the osteotomy. A total of two osteotomies in the right side and an osteotomy in the left side of Sprague Dawley rat will be performed, where a screw shaped SMB implant and a control (mock surgery, just drilling and no implant) in the right side and a screw shaped MB implant in the left site are placed. The implants will remain for 4 or 8 weeks *in vivo*. The animals will be sacrificed and the implants and surrounding bone will be retrieved and examined for gene expression levels, histology, and bone mechanical properties. Animal studies will be performed under protocols approved by the New York University School of Medicine (protocol #090504-03). 10 rats × 2 time points = 20 animals. Further experiments may be needed based on the results of the proposed experiments.

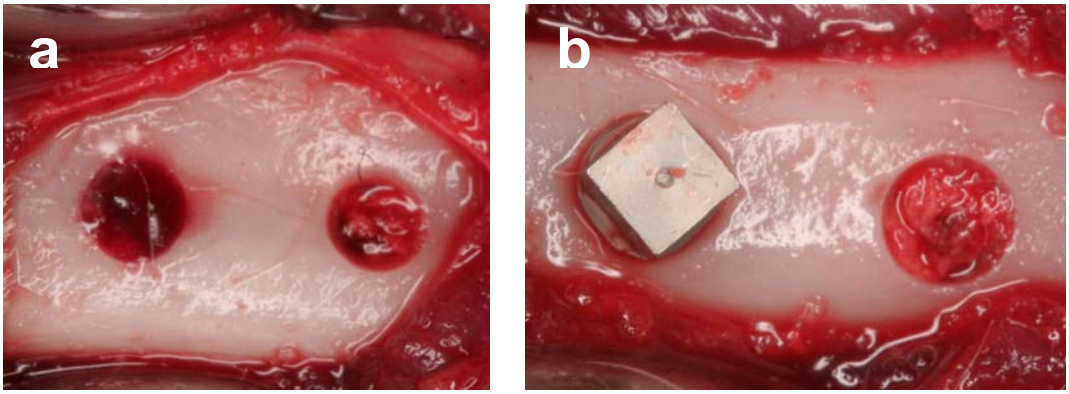

Fig. 3. (a) A total of two osteotomies will be performed in the right side of Sprague Dawley rat. (b) A screw shaped SMB implant and a control (mock surgery, just drilling and no implant) will be placed in the right side.

Sacrifice

At 4 and 8 weeks after implant placement surgery, animals will be sacrificed using carbon dioxide. The femurs containing implants will be then harvested and stored in Trizol Reagent (Invitrogen, Carlsbad, CA) at -20ºC and followed their protocol for gene expression analysis. For analysis of histology and bone mechanical properties, femurs will be transferred to 10% neutral buffered formalin at a volume 10× that of the specimen.

Gene expression analysis

After the femurs will be harvested and all soft tissues will be removed, the bone samples (the area surrounding the implant) will be stored in PULSE Tubes and total RNA will be isolated separately from these samples using a new technology, pressure cycling technology. Target-specific PCR primers for alkaline phosphatase (ALP), bone sialoprotein (BSP), type II collagen (Col-II), osteocalcin (OCN), osteopontin (OPN), runt-related transcription factor 2 (RUNX2), and β-actin (as for an internal control)

will be designed using the ProbeFinder assay design software. cDNA will be synthesized from 1 μg of total RNA for each sample using reverse transcriptase (Roche). Reactions for the 480 LightCycler will be performed in 20 μl reaction volumes for the genes encoding ALP, BSP, Col-II, OCN, OPN, RUNX-2 and β-actin using 1 μl of cDNA under the following conditions: 95ºC for 5 min, 50 cycles for 95ºC for 10 sec, 60ºC for 15 sec, and 72ºC for one sec. The method used for obtaining quantitative data of relative gene expression will be the comparative Ct method (also as known the $2^{-\Delta\Delta Ct}$ method). Triplicates of each data point will be averaged, and the mean values will be used for statistical analysis.

Nanoindentation

Nanoindentation tests evaluate the biomaterial properties of bone tissue. The harvested bone will be embedded in PMMA (8.00590.2500 Merk) and the blocks will be cut into two pieces transversally through the middle of the implant using the diamond wire saw. The face of one half of the transverse cuts will be polished and finished with a 0.25 μm diamond solution. Following this, the specimens were kept at –4°C. Specimens will be slowly thawed at 7°C overnight and then warmed to room temperature prior to nanomechanical testing. In this test, force–displacement data of a pyramidal diamond indenter that is pressed into the bone material will be recorded. Measurements will be performed with a nano-hardness tester (NHT, CSM Instruments, Peseux Switzerland). The tests will include 5 indents on the trabecular bone situated around the implant and 5 indents on the trabecular bone situated at 200 μm from the implant. Cortical bone next to the implant will be also tested, with 5 indents performed on the cortical bone next to the implant. Specimens will be kept in a saline solution before and after the testing. Indents will be set to a 900 nm depth with an approximate strain rate of $\varepsilon = 0.066$ 1/s for both loading and unloading. At maximal load, a 5-s holding period will be applied. The limit of the maximum allowable thermal drift will be set to 0.1 nm/s.

Histology

The samples will be kept in 10% buffered formalin solution for 24 h, washed in running water for 24 h, and gradually dehydrated in a series of alcohol solutions ranging from 70% to 100% ethanol. Following dehydration, the samples will be embedded in a methacrylate-based resin (Technovit 9100, Kulzer & Co., Wehrheim, Germany) according to the manufacturer's instructions. The blocks will be cut into slices (~300 mm thickness) aiming the center of the implant following its long axis with a precision diamond saw (Carbimet disks, Buehler, Lake Bluff, IL), glued to acrylic plates with an acrylate-based cement, and a 24 h setting time will be allowed prior to grinding and polishing. The sections will be reduced to a final thickness ~30 mm with a series of SiC abrasive papers (Isomet 2000, Buehler) (400, 600, 800, 1,200, and 2,400 grits) in a grinding/polishing machine (Metaserv 3000, Buehler) under water irrigation. The sections will be then toluidine blue stained and referred to optical microscopy evaluation.

Statistical analysis

Statistically significant differences ($p < 0.05$) between the various groups will be measured by ANOVA and SNK post hoc or Kruskal–Wallis non-parametric procedure, followed by Mann–Whitney U-test for multiple comparisons based on the normal distribution and equal variance assumption test. All statistical analyses will be carried out using SPSS statistical software package (SPSS, Chicago, IL).

Project time line

The results of this proposal will provide preliminary data and publications which will support subsequent grant applications. We will propose to test several different candidates of novel implant surfaces using this model. These approaches may lead to an improvement in the current clinical treatment of missing teeth. This could lead to a shorter, more predictable, and affordable treatment for partially and fully edentulous patients. Anticipated completion date: December 2012

1. Preparation of implants and implant surgery: January 2012 – February 2012
2. Real-time PCR analysis: February 2012– April 2012
3. Nanoindentation & histological analysis: May 2011 – September 2012
4. Preparation of reports, publications, and grants: October 2012 – December 2012

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