Comparing Initial Wound Healing and Osteogenesis of Porous Tantalum Trabecular Metal and Titanium Alloy Materials

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Porous tantalum trabecular metal (PTTM) has long been used in orthopedics to enhance neovascularization, wound healing, and osteogenesis; recently, it has been incorporated into titanium alloy dental implants. However, little is known about the biological responses to PTTM in the human oral cavity. We have hypothesized that, compared with conventional titanium alloy, PTTM has a greater expression of genes specific to neovascularization, wound healing, and osteogenesis during the initial healing period. Twelve subjects requiring at least 4 implants in the mandible were enrolled. Four 3×5mm devices, including 2 titanium alloy tapered screws and 2 PTTM cylinders, were placed in the edentulous mandibular areas using a split-mouth design. One device in each group was trephined for analysis at 2 and 4 weeks after placement. RNA microarray analysis and ingenuity pathway analysis were used to analyze osteogenesis gene expression and relevant signaling pathways. Compared to titanium alloy, PTTM samples exhibited significantly higher expressions of genes specific to cell neovascularization, wound healing, and osteogenesis. Several genes—including bone morphogenic proteins, collagens, and growth factors—were upregulated in the PTTM group compared to the titanium alloy control. PTTM materials may enhance the initial healing of dental implants by modifying gene expression profiles.

Key Words: gene array, osseoincorporation, tantalum, trabecular metal, wound healing

INTRODUCTION

Tantalum (Ta) is a very rare transitional metal with high corrosion resistance properties. The significant reactivity to oxygen and rarity of Ta had limited its applications in the medical and dental fields until the last 2 decades.[1–3] Porous tantalum trabecular metal (PTTM) was commercially developed in the early 1990s and has been widely used in orthopedic implants for over 2 decades.[2] More recently, it has been incorporated into titanium (Ti) alloy dental implants.[1,2,4] PTTM combines the physical strength and biocompatibility of tantalum with a three-dimensional, open-cell porous dodecahedron vitreous carbon internal framework.[2,5,6] This theoretically allows up to 70%–80% greater bone-implant contact.[1] The three-dimensional porosity of PTTM has been shown to enhance neovascularization and osteogenesis.[1,2,4,7–9] This allows for a third dimension of bone-to-implant ongrowth and ingrowth known as osseoincorporation, as opposed to the two-dimensional bone ongrowth known as osseointegration of traditional titanium implants.[1,8,10] By facilitating new osseous tissue formation inside its highly porous structure, PTTM may strengthen immediate and early loading of implants by enhancing vascular stem cell proliferation and osteoblastic differentiation. While PTTM has been used for over 2 decades in orthopedic implants throughout the body, PTTM-host responses in the oral cavity are not well understood. The few published clinical studies have reported promising clinical results,[11–14] but specific gene expression or molecular insight into this success is not known. This study hypothesized that PTTM would enhance initial healing in the

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oral cavity through osteogenesis-specific gene expression as compared to conventional Ti alloy.

Materials and Methods

Study population

Subjects with symmetrical edentulous areas in the mandible that require placement of at least 4 implants (2 implants on each side of the mandible) were recruited through the UNC healthcare system. We selected only subjects with healed native bone without history of grafting. Subjects with active periodontal disease were excluded from the study. We applied a prospective split-mouth design to this study. Titanium alloy or PTTM devices were randomly placed in the left or right side of the edentulous areas in the mandible using block randomization. A randomization chart was generated prior to the recruitment, and the surgeons were told at the time of device placement where the placements should go. The surgical operator was given the cylinders after all osteotomy sites were prepared. A total of 13 subjects were recruited, and signed informed consent was obtained. Out of 13 subjects, 1 subject was withdrawn due to insufficient alveolar bone after radiographic examination. Table 1 describes the inclusion and exclusion criteria. There are currently only a handful of studies examining the gene expression profiles for retrieved implanted devices in the oral cavity.[15,16,17] These studies had between 9 and 11 subjects total. Our goal was to have 12 subjects total in our study. The primary outcome measure of the study was the differential gene expression, while the secondary outcome measure was the histological bone-implant contact.

Clinical treatments

Two 3 $\times$ 5 mm devices were evaluated in this study: a PTTM cylinder (test) and a tapered Ti alloy screw (control). The latter was a self-tapping design prepared with a commercially available grit-blasted surface (MTX, Zimmer Biomet Dental, Palm Beach Gardens, Fla). Each subject was screened clinically and radiographically. Cone-beam computed tomography (CBCT) scans were used to examine the pre-existing alveolar bone. The CBCT scans were reviewed by one of the implant surgeon investigators (S. Bencharit or T. Morelli). Simplant 16 (Dentsply, York, Pa) or 360 dps (360imaging, Atlanta, Ga) implant planning software was used to determine if there was sufficient bone to accommodate a 3 $\times$ 5 mm study device. A minimal width of 7 mm and height of 8 mm for each implant site were used as inclusion criteria to ensure that the study device could be placed without bone dehiscence or adversely affecting adjacent anatomical structures. At each surgical visit, subjects were asked to rinse their mouths with 0.12% chlorhexidine gluconate prior to the procedure. Surgery was performed under local anesthesia administered via bilateral inferior alveolar nerve blocks, long buccal nerve blocks and local infiltration of the surgical sites. Full thickness flaps were raised in all sites. A split-mouth design was used. Four osteotomy sites were located and prepared based on the preoperative CBCT scans, implant planning software, and a surgical guide. Two Ti alloy tapered screws and two PTTM cylinders were placed in each edentulous site (Figure 1a and b). Since the Ti alloy device was a self-tapping tapered screw, a 2.3 mm drill was used and the Ti alloy device was self-tapped into place. However, since the PTTM device was a straight cylinder, a 3 mm drill was used to place the device. The PTTM device was press-fitted in place. The Ti alloy devices were placed on one side (right or left), and the PTTM devices were placed on the opposite side. The top of each device was placed at the bone level. A 5 mm diameter resorbable collagen membrane (Biomend, Zimmer) was placed on top of each device. In this study, our primary goal was to examine the bone-implant healing; therefore, the barrier membrane was placed to minimize the effect of soft tissue ingrowth. A combination of continuous interlocking and interrupted suturing techniques with 4.0 chromic gut sutures were used to ensure primary closure and hemostasis. The subjects were instructed to continue using a 0.12% chlorhexidine rinse until the completion of the study (a total of about 6 weeks, 2 weeks additional after the final surgery). For subjects who were wearing an interim denture, the denture was relined with tissue conditioner (COE-Comfort, GC America, Allsip, Ill). The tissue condition was changed every 2 to 4 weeks until the subject exited the study. At 2 and 4 weeks after placement, 1 device from each group was removed using a 4 mm diameter trephine drill (Salvin Dental Specialties, Charlotte, NC). The posterior sites were done at 2 weeks and the anterior sites were done at 4 weeks. All samples were subjected for analysis of osteogenesis gene expression analyses and relevant signaling pathways. In addition, selected samples in the 4-week group were also subjected to histological analysis. After removal of each device, the osteotomy site was further prepared for definitive implant placement. Conventional Ti alloy dental implants (Tapered Screw-Vent, Zimmer) were placed in each site according to the manufacturer's specifications. Bone allograft (Puros Cancellous Particulate, Zimmer) and an appropriate resorbable collagen membrane (eg, Biomend, Zimmer) were used as needed if there was bone dehiscence around the dental implant. Subjects were then reappointed at least once more 2 weeks after removal of the study devices and placement of the definitive dental implants. After the clinical operator determined that the subjects had appropriate soft tissue healing, subjects then exited the study and were referred back to their restorative dentists to fabricate appropriate definitive prostheses.

Osteogenesis PCR array analysis, IPA analysis, and statistical analysis

After the sample was harvested, it was placed in RNA stabilization solution (RNAlater, ThermoFisher Scientific, Carlsbad, Calif) for 24 hours. The samples were then immersed in liquid nitrogen and stored at -80°C until analysis.[18] The frozen samples were then ground and homogenized. Total RNA extraction was performed using miRNeasy Mini Kit (Qiagen, Hilden, Germany). This allowed quantitative comparison of RNA expression levels with housekeeping genes. The RNA quality and purity were analyzed by spectrophotometer using NanoDrop (Thermo Scientific, Wilmington, Del). Gene expression during human osteogenesis was confirmed by a polymerase chain reaction (PCR) array-based method (TaqMan microarray

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Table 1

Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Subjects with edentulous spaces in the mandible requiring placement of at least 4 dental implants and the edentulous alveolar ridge of at least 7 × 8 mm minimal cross-sectional width.	Subjects with other diseases known to impair wound healing. Active infectious disease. Use of any medication known to affect periodontal status within 1 month prior to the initial examination. Current smokers or history of smoking within the last 2 years. Pregnant women. Blood disorders, chemotherapy or radiation therapy.

panels, ThermoFisher Scientific) and analysis was performed on the Qiagen/SAB Biosciences website (https://www.qiagen.com).

The osteogenesis array panel includes 84 genes related to skeletal development, bone mineral metabolism, extracellular matrix (ECM) molecules, cell adhesion molecules, growth factors, and transcriptional factors. Housekeeping genes include GAPDH, ACTB. A cut-off point of 35 cycles was applied. Data were analyzed online through the use of Ingenuity Pathway Analysis (Ingenuity System, Inc, Redwood City, Calif) to interpret the data in the context of biological processes, pathways and networks. Both up- and down-regulated identifiers were defined as value parameters for the analysis. Focus genes, mapped to corresponding gene objects in the Ingenuity Pathways Knowledgebase (IPKB), found in IPA, were used to generate biological networks.

Significance of the bio-functions and the canonical pathways were tested by the Fisher Exact test *P* value, calculated online from the right-tailed Fisher Exact Test. The IPA calculated the number of proteins in the pathway that met cutoff criteria for differential expression versus the number that did not. This was used to generate a ratio of the pathways. An independent biostatistician reviewed the results and statistical analyses.

Histologic analysis

Trephined samples collected from 6 subjects, including 2 test cylinders per subject, were processed for histological analysis. The samples were transferred to 0.1 M Cacodylate buffers, pH 7.4, for several hours to overnight. Dehydration was started with an ethanol series: 50%, 70%, and 95% ethanol in distilled water for 10 minutes each. They were then transferred into absolute ethanol for 2 rinses of 20 minutes each. The samples were infiltrated with a 50:50 mixture of Polybed resin (Polysciences, Inc, Warrenton, Pa) and absolute ethanol for 6 to 12 hours. The samples were then embedded with several changes of pure resin into BEEM capsules and cured overnight at 65°C. The orientation of the samples during embedment was carefully maintained to facilitate cross-sectioning of the implants. Cured resin blocks containing the implants were removed from the polyethylene capsules and ready for sectioning using a diamond knife. Sections were made using a diamond saw. Undecalcified sections were obtained by cutting the implants longitudinally. The sections were stained with 1% toluidine blue. A representative section of the midportion of the implant, closest to the midline of the device specimen, was used for the histometric analysis. The sections were masked and presented for analysis, which were performed by one calibrated examiner (S. Barros). Images were obtained using Zeiss microscope (Zeiss CLSM 700) and bone-implant contact (BIC) measurements[5] obtained by linear measurement of direct BIC on the threads using Image Pro (Media Cybernetics, Inc, Silver Spring, Md) compared using paired *t* test statistical test.

Ethics

The study protocol was approved by the University of North Carolina (UNC) Human Research Ethic Committee (IRB # 11-2539).

Results

Patient demographic information is summarized in Table 2. A total of 12 subjects completed all visits. The first subject was enrolled in January 2013, and the last subject was enrolled in May 2014. The last subject completed a final visit in February 2015. One subject was withdrawn because of insufficient alveolar bone. A total of 12 subjects participated in all clinical treatments. After retrieval, the PTTM test devices appeared to have more tissues adhering to their surfaces than did the Ti alloy controls (Figure 1c). The hard and soft tissues surrounding the Ti alloy device were non-adherent and often separated from the device (Figures 1c through g), especially when retrieval was performed at the 2-week period. Radiographically at 2 weeks, there was clear radiolucency around the Ti alloy device, which was indicative of bone remodeling (Figure 1d). This radiolucency was absent in the PTTM group (Figure 1d). Retrieval of PTTM devices was more difficult compared to the Ti alloy devices, especially at the 2-week period when the PTTM devices appeared to be already fused with adjacent bone. As the devices were being removed, we noticed differences between the 2 materials in terms of their physical adherence to the peri-device bone. At 2-week time point, almost all Ti alloy devices were immediately separated from the bone; however, all PTTM devices adhered to the bone very well. At the 4-week time point, only about half of the Ti alloy devices adhered, while all PTTM devices appeared to adhere very well to the surrounding bone.

Gene expression profiles of PTTM and control Ti alloy devices at 2 and 4 weeks were retrieved and compared. Fold regulation representing the fold changes in a biologically meaningful way is described in Table 3 based on the gene function grouping. Genes specific to osteoblastic differentia-

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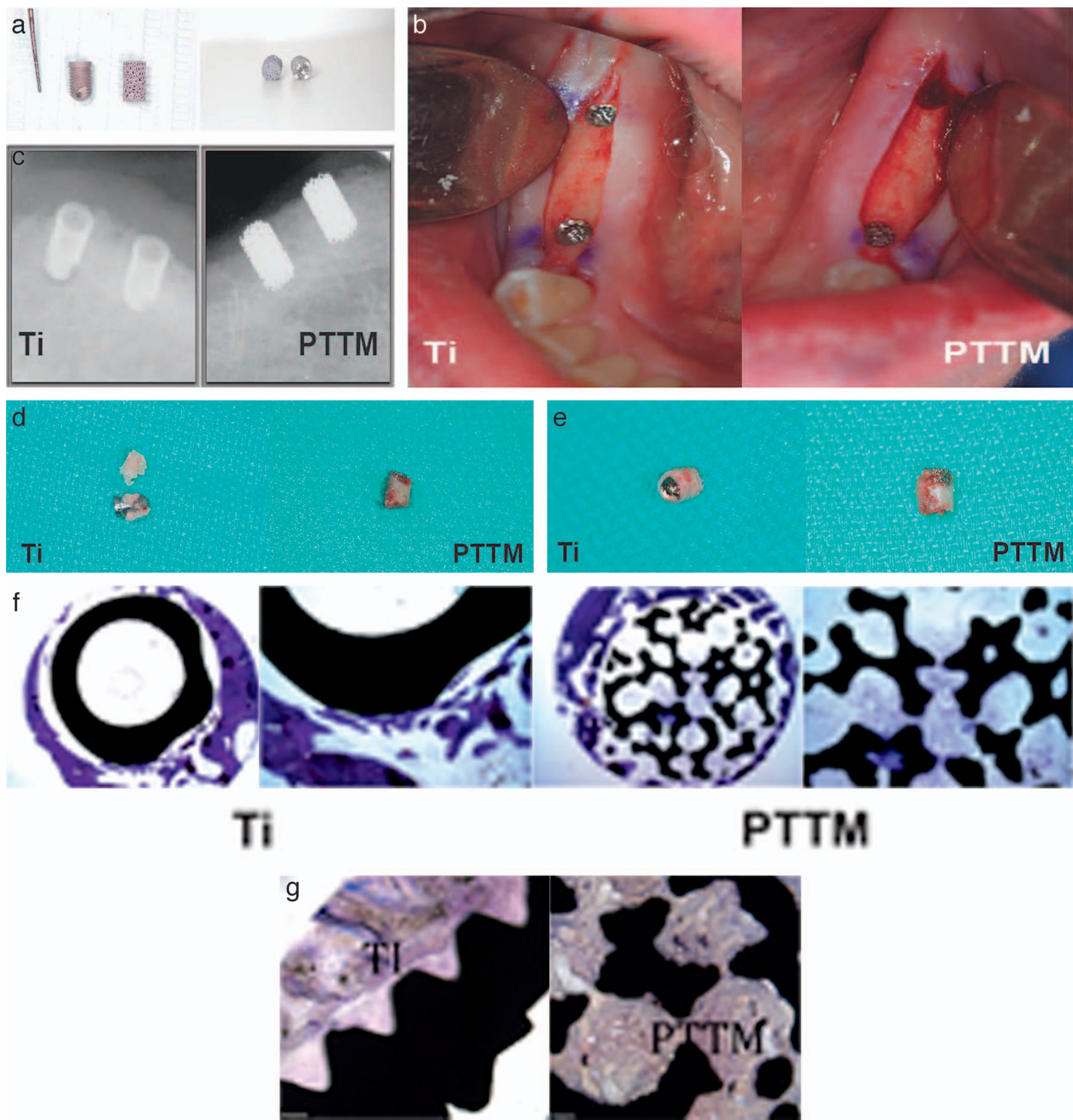

Figure 1. Surgical placement and retrieval of Ti alloy and porous tantalum trabecular metal (PTTM) devices. (a) Ti alloy and PTTM devices. (b) Placement of the control Ti alloy and PTTM devices. (c) Periapical radiograph taken 2 weeks after the placement showing the radiolucent bone remodeling around the Ti alloy devices. (d) Surgical retrieval of devices at 2 week post-placement visit showing bone adhering to the PTTM but not the Ti alloy devices. (e) Surgical retrieval of devices at 4 week post-placement visit. (f) Histological sections of Ti alloy and PTTM devices. (g) High resolution photomicrographies illustrating the histologic aspects observed in the bone implant contact.

tion—including bone morphogenic proteins (BMPs), alkaline phosphatase (ALPL), Runt-related transcription factor 2 (RUNX2), Distal-less Homeobox 5 (DLX-5), bone gamma-carboxyglutamate (Gla) protein (osteocalcin), as well as collagens—were upregulated in the PTTM group compared to Ti alloy control at 2 and 4 weeks (Table 3; Figure 2). The expressions of most osteoblast specific genes were distinctly higher at 4 weeks (Figure 2a), which seemed to coincide with the upregulation of collagens (Figure 2b). In addition to the osteoblast-specific genes, growth factors associated with wound healing and neovascularization were also upregulated in the PTTM group compared to Ti alloy control (Figure 2c):

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Table 2

Demographic data of included subjects

	#	Age Range, y	Mean
Sex			
Male	5	60–75	67
Female	7	39–67	55.6
Total age range		39–75	59.4
Race/ethnicity			
Caucasian, non-Hispanic	9		
African American, non-Hispanic	2		
Asian, non-Hispanic	1		

Table 3

Gene expression differences between porous tantalum trabecular metal and Ti alloy

	Fold Regulation*	
	2 Weeks	4 Weeks
Bone-specific growth factors		
ALPL	**2.1522**	**3.7804**
BMP1	**3.2773**	−1.2169
BMP2	**2.9811**	*−2.9515*
BMP3	**3.5125**	**10.5422**
BMP4	**6.3606**	**2.2148**
BMP5	**5.275**	**10.4561**
BMP6	1.6577	−1.6081
BMP7	**3.8526**	**6.7917**
BMPR1A	1.0962	**5.9355**
BMPR1B	1.3939	1.6524
BMPR2	1.3006	**2.8905**
DLX5	**3.5615**	**11.4817**
RUNX2	−1.4249	**10.7108**
Collagen-specific growth factors		
Col X _1	1.9532	−1.641
Col XIV _1	**2.94**	1.2618
Col XV _1	−1.2177	*−2.0577*
Col I _1	1.4837	1.6005
Col I _2	1.865	**5.2706**
Col III _1	1.2304	1.0328
Col V _1	1.9352	**5.8948**
Non–bone-specific growth factors		
EGF	1.8693	**2.5645**
EGFR	1.7808	**4.4134**
FGF1	1.0299	**5.255**
FGF2	−1.0287	**4.7315**
FGFR1	1.0911	**7.5237**
FGFR2	1.1089	**10.2938**
FN1	−1.0287	**3.2572**
GLI1	**2.312**	**12.7672**
ICAM1	−1.1982	**2.638**
IGF1	1.0157	**15.3149**
IGF1R	1.4004	**10.6861**
IGF2	1.1587	**15.0029**
TGFB1	−1.37	**2.2872**
TGFB2	−1.5449	**7.0424**
TGFB3	−1.0528	**4.5197**
TGFBR1	−1.1335	**4.1544**
TGFBR2	−1.0359	**5.0422**
TNF	−1.0029	1.1149
TNFSF11	−1.9375	1.5349
VEGFA	−1.1735	**3.1291**
VEGFB	1.4599	**2.2684**
VGFR1 (FL1)	−1.6984	**6.2392**
Genes with notably high expression		
BMP-3b/BMP3B (GDF10)	1.0275	**133.2569**
Col II _1	1.5009	**44.4846**
BGLAP (Osteocalcin)	**2.9605**	**55.5571**

*Bold represents statistical differences of up-regulated markers. Italics represents statistical differences of down-regulated markers.

insulin growth factors (IGFs), epithelial growth factors (EGFs), and vascular endothelial growth factors (VEGFs). Certain proteins that were upregulated in notably high levels included BMP3b, Col II, and Osteocalcin (Figure 2d). IPA suggested that signaling pathways associated with osteogenesis and bone matrix maturation were upregulated in PTTM compared to Ti alloy samples.

The histological examinations of PTTM and Ti alloy devices confirmed the clinical observations. The bone contact (Table 4) at 2 weeks was 57% higher in the PTTM test group as compared to the Ti alloy control group (Figure 1f and g). There was more initial soft tissue healing around the PTTM than in the Ti alloy devices. More importantly, bone remodeling occurred both in the center and peripheral areas of the PTTM devices. The highest mean values for BIC were observed in tantalum samples. The tantalum surface promoted an enhanced BIC (40.06%) compared with titanium (21.52%) ($P < .04$).

Discussion

This study may be the first to examine the initial healing of PTTM material at the genetic level in the human oral cavity. The concept of "osseoincorporation," which refers to 3-dimensional bone growth onto (osseointegration) and into the implant material itself, has recently been introduced to implant dentistry.[1] Study findings suggest that PTTM material facilitates neovascularization and promotes bone ingrowth and neoformation inside the implant. This bone ingrowth has been shown repeatedly at the histologic level in retrieved orthopedic implants,[19,20] as well as in the jaws of animals.[5,10,21] The current results highlight clinical bone ingrowth into PTTM as early as 2 weeks after placement. These findings are similar to those of other studies in canine models that found faster bone ingrowth and a higher level of bone contact and neovascularization histologically in the PTTM sections when compared with Ti alloy sections of dental implants.[5,10,22] Bone healing of PTTM in the present study appeared to occur from the inside of the material compared to the external surfaces of the Ti alloy devices, which was comparable to findings in animal models.[5,10,21] Additionally, neovascularization also occurred within the PTTM material.

Therefore, the current study results clinically and histologically supported the concept of osseoincorporation. While this study was the first to show initial bone healing at the genetic level in the human oral cavity, the histological and clinical results are only physical examinations and not novel. The main study goal of the present study was examination of cellular signaling through analyzing gene expression of PTTM compared to Ti alloy control to understand how PTTM may modulate bone healing. In other words, this study examined how PTTM altered signaling molecules during initial healing of 2- and 4-week periods. These periods were chosen because it was expected that maximal expression of genes associated with initial healing would occur immediately after dental implants

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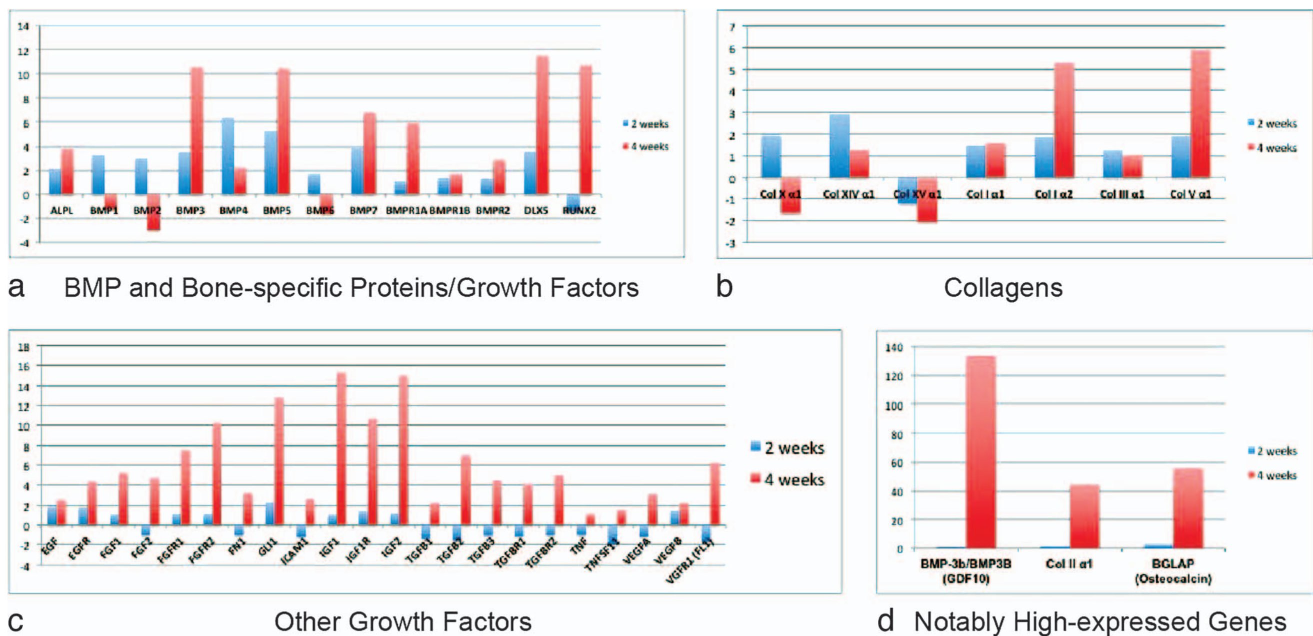

FIGURE 2. Gene expression profiles of selected genes comparing porous tantalum trabecular metal to Ti alloy at 2 weeks (blue) and 4-week (red) visits. (a) Bone morphogenic protein and bone specific proteins. (b) Collagens. (c) Growth factors. (d) Genes with notably high expression. BMP indicates bone morphogenic proteins.

were placed. This was achieved by specifically examining gene expression specific to osteoblastic differentiation, wound healing, and neovascularization.

In support of physical bone ingrowth, the gene expression profile suggested a faster activation of osteoblastic or bone-specific genes (Figure 2a). The signaling pathways pointed out a simultaneous stimulation of bone matrix, vascularization, wound healing, and osteogenesis (Figure 2). The increase in expression of these genes in the PTTM group compared with Ti alloy was initiated at the 2-week time point, and the results appeared more pronounced at the 4-week time point (Figure 2 a through d). Growth factors such as EGF, IGF, VEGF and others suggest that the stimulation of neovascularization was faster in the PTTM group than in the Ti alloy group. Wound healing and osteogenesis signaling molecules also have similar expression patterns. For instance, RUNX2, DLX-5, as well as most BMPs suggest early and more pronounced activation of osteoblastic differentiation. In the PTTM group, the gene expression pattern of several collagen proteins and matrix maturation proteins—such as bone gamma-carboxyglutamic acid-containing protein, also known as osteocalcin and alkaline phosphatase—suggest faster formation of ECM and enhanced mineralization of the ECM. The present results are similar to the studies comparing different Ti alloy implant surface modifications.[15,23] These gene array studies of Ti alloy surface modification[15,23,24] as well as bone healing of Ti alloy implants[25] up to 2 weeks' time have shown an improvement of 1.5- to 2-fold higher expression of genes. However, the present PTTM results were significantly more pronounced with higher fold regulation difference in almost all genes when PTTM and Ti alloy were compared (Table 2). This suggests that PTTM altered gene expression during initial healing in a much broader and higher scale than just surface modification. This signaling pattern is similar to the osteogenesis of human adipose-derived stem cells a three-dimensional collagen culture.[26] PTTM material itself did not seem to have much of an effect on osteoblasts in cell culture[27]; however, the authors thought that the healing of PTTM in humans was different because of the simultaneous activation of neovascularization, wound healing, and osteogenesis. Therefore, it is proposed that the 3-dimensional (3D) structure of the PTTM itself may have contributed to this unique healing phenomenon of osseoincorporation.[1,2] In fact, it has been shown that 3D culture can enhance global gene expression for in vitro embryonic stem cells[28] as well as osteoblastic differentiation from elderly female patients.[29] The 3D structure of PTTM has been shown to promote bone regeneration when used as a stem cell carrier.[30,31]

Based on the present results in healthy humans, we can present an osseoincorporation signaling model of PTTM similar to the osseointegration model summarized by Nishimura.[32] The IPA results suggest that PTTM promoted osteoblast or bone-specific genes, for instance, BMP2, BMP5, and DLX-5 (Figure 3a). This in turn mediated the matrix maturation, presented as high expression of several collagens (Figure 3b). The authors present a new model (Figure 3c) that suggests that PTTM initially stimulates osteogenesis through activation of bone-specific transcriptional and growth factors, such as BMP1, 2, 3, 4, 5, and

TABLE 4

Bone-implant contact mean values (%) comparing titanium and tantalum surfaces at 4 weeks post-implant placement*

Groups	Mean % ± SD
Titanium surface	25.55 ± 0.67
Tantalum surface	40.06 ± 1.2

*Paired t test, P value = .042

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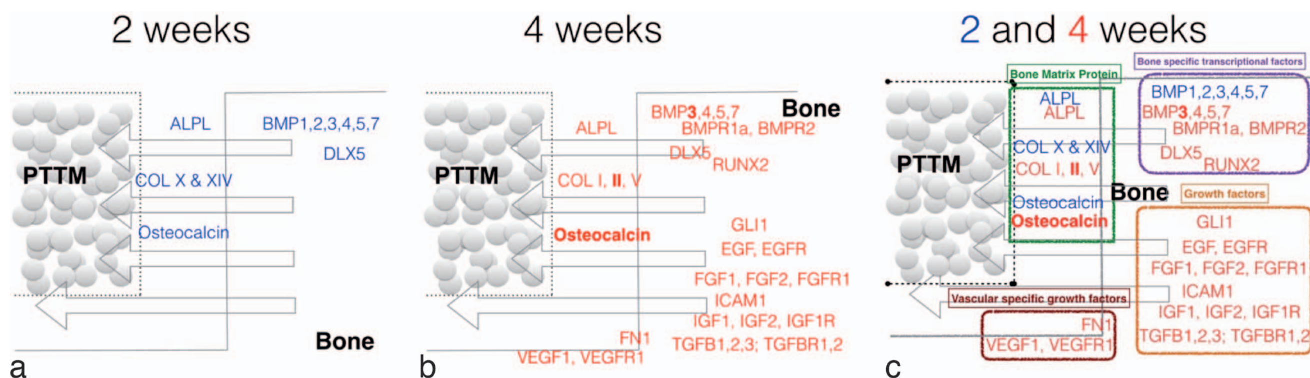

Figure 3. Proposed signaling model for porous tantalum trabecular metal (PTTM) and osseoincorporation. (a) Genes upregulated associated with PTTM at 2-week visit. (b) Genes upregulated associated with PTTM at 4-week visit. (c) Combined genes upregulated associated with PTTM at 2- and 4-week visits. Note that notably highly expressed genes are in bold.

7, as well as DLX-5. This activation enhances matrix maturation and mineralization seen as upregulation of osteocalcin, alkaline phosphatase, Collagen X (Col X), and Collagen XIV. This suggests that the PTTM devices have higher activation of bone specific genes as early as 2 weeks compared to Ti alloy. The differential expression of different types of collagen suggests that the bone around the PTTM device is more mature than the one around the Ti alloy. For example, Col X, which is almost 2-fold greater in PTTM at 2 weeks, represents hypertrophic cartilage and is essential for later stages of chondrocyte differentiation. This suggests a more mature bone healing around PTTM compared to Ti alloy. Col X and Col II, found mainly in cartilage, play an important role in endochondral ossification. Their upregulation associated with PTTM suggests different mechanism in bone healing and maturation compared to Ti alloys. Note also that these two collagens have not been reported as associated with titanium dental implant healing. Matrix maturation via collagen and mineralization seen by osteocalcin and alkaline phosphatase also took place at the 2-week time point. The PTTM-associated gene activation and enhancement was more dramatic compared to Ti alloy at the 4-week time point (Figure 3d). The activation of osteoblastic/bone specific transcriptional/growth factors in combination with other growth factors enhanced the vascularization and bone matrix maturation. At the 4-week time point, BMPs and their receptors—such as BMP3, BMP4, BMP5, BMP7, BMPR1, and BMPR2—were activated. This BMP activation coincided with other transcriptional factors related to osteogenesis, including RUNX2 and DLX-5. While the bone-specific growth factors were upregulated, an additional upregulation of a wide-range of growth factors—including Zinc finger protein GLI1 (also known as glioma-associated oncogene), EGFs, fibroblast growth factors (FGFs), IGFs, intercellular adhesion molecule (ICAM), and transforming growth factor beta (TGF-β) were also observed. The upregulation of these growth factors represented the enhancement of general wound healing in PTTM when compared to Ti alloy. Perhaps as the consequence of this growth factor upregulation, this study also found faster neovascularization through VEGFs and fibronectin 1 (FN1) as well as maturation and mineralization of the matrix through collagens, alkaline phosphatase, and osteocalcin.

We recognize certain limitations of this study. First, while the device design was an attempt to mimic the Ti and PTTM portion of commercially available dental implants, there were differences between the Ti and PTTM treatment in terms of the osteotomy site preparation (2.3 mm in Ti vs 3 mm in PTTM) and the nature of tapered-screw type and press-fitting type devices. Higher bone compression of Ti devices compares to the PTTM ones may potentially result in adverse gene expression, resulting in initial bone resorption and higher inflammatory responses.[33,34] Their differences can be one of the confounding factors that need further evaluation. For instance, one should compare the same osteotomy preparation and configuration of Ti and PTTM devices. Second, the study design only allows us to examine 2 time points in the initial stage of the healing. The results may not reflect longer periods of implant healing.

In summary, this study supports the concept of osseoincorporation where PTTM material allows bone ongrowth and ingrowth by promoting simultaneous neovascularization, wound healing, and osteogenesis when compared to Ti alloy in the early stage of implant healing. The authors propose that the 3D structure of PTTM enhances global gene expression and osteogenesis.

Conclusions

Based on the limitations of this study, PTTM material enhances initial healing compared to Ti alloy in the mandible of healthy subjects. Further studies in other areas of the oral cavity (such as the maxilla) and with different patient population (such as diabetics or patients with osteopenia) need further examination to determine if PTTM will have similar effects in patients with compromised implant healing due to systemic conditions or bone quality/quantity problems.

Abbreviations

ALPL: alkaline phosphatase
BIC: bone-implant contact
BMP: bone morphogenic protein
BMPR: bone morphogenic protein receptor
CBCT: cone-beam computed tomography
DLX-5: Distal-less Homeobox 5

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ECM: extracellular metrix
EGF: epithelial growth factors
FN1: fibronectin 1
Gla: bone gamma-carboxyglutamate protein or osteocalcin
IGF: insulin growth factors
IPA: ingenuity pathway analysis
PCR: polymerase chain reaction
PTTM: porous tantalum trabecular metal
RUNX2: Runt-related transcription factor 2
VEGF: vascular endothelial growth factor

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References

1. Bencharit S, Byrd WC, Altarawneh S, et al. Development and applications of porous tantalum trabecular metal-enhanced titanium dental implants. *Clin Implant Dent Relat Res*. 2014;16:817–826.
2. Levine BR, Sporer S, Poggie RA, Della Valle CJ, Jacobs JJ. Experimental and clinical performance of porous tantalum in orthopedic surgery. *Biomaterials*. 2006;27:4671–4681.
3. Branemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg*. 1969;3:81–100.
4. Bencharit S, Byrd WC, Hosseini B. Immediate placement of a porous-tantalum, trabecular metal-enhanced titanium dental implant with demineralized bone matrix into a socket with deficient buccal bone: a clinical report. *J Prosthet Dent*. 2015;113:262–269.
5. Kim DG, Huja SS, Tee BC, et al. Bone ingrowth and initial stability of titanium and porous tantalum dental implants: a pilot canine study. *Implant Dent*. 2013;22:399–405.
6. Bobyn J, Pilliar R, Cameron H, Weatherly G. The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. *Clin Orthop Relat Res*. 1980;150:263–270.
7. Liu Y, Bao C, Wismeijer D, Wu G. The physicochemical/biological properties of porous tantalum and the potential surface modification techniques to improve its clinical application in dental implantology. *Mater Sci Eng **C** Mater Biol Appl*. 2015;49:323–329.
8. Bobyn JD, Stackpool GJ, Hacking SA, Tanzer M, Krygier JJ. Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *J Bone Joint Surg Br*. 1999;81:907–914.
9. Hacking S, Bobyn J, Toh K, Tanzer M, Krygier J. Fibrous tissue ingrowth and attachment to porous tantalum. *J Biomed Mater Res*. 2000;52: 631–638.
10. Lee JW, Wen HB, Battula S, Akella R, Collins M, Romanos GE. Outcome after placement of tantalum porous engineered dental implants in fresh extraction sockets: a canine study. *Int J Oral Maxillofac Implants*. 2015; 30:134–142.
11. Papi P, Jamshir S, Brauner E, et al. Clinical evaluation with 18 months follow-up of new PTTM enhanced dental implants in maxillo-facial post-oncological patients. *Ann Stomatol (Roma)*. 2014;5:136–141.
12. Schlee M, Pradies G, Mehmke WU, et al. Prospective, multicenter evaluation of trabecular metal-enhanced titanium dental implants placed in routine dental practices: 1-year interim report from the development period (2010 to 2011). *Clin Implant Dent Relat Res*. 2015;17:1141–1153.
13. Schlee M, van der Schoor WP, van der Schoor AR. Immediate loading of trabecular metal-enhanced titanium dental implants: interim results from an international proof-of-principle study. *Clin Implant Dent Relat Res*. 2015;17(suppl 1):e308–e320.
14. Brauner E, Guarino G, Jamshir S, et al. Evaluation of highly porous dental implants in postablative oral and maxillofacial cancer patients: a prospective pilot clinical case series report. *Implant Dent*. 2015;24:631–637.
15. Donos N, Hamlet S, Lang NP, et al. Gene expression profile of osseointegration of a hydrophilic compared with a hydrophobic microrough implant surface. *Clin Oral Implants Res*. 2011;22:365–372.
16. Bryington M, Mendonca G, Nares S, Cooper LF. Osteoblastic and cytokine gene expression of implant-adherent cells in humans. *Clin Oral Implants Res*. 2014;25:52–58.
17. Thalji G, Cooper LF, Nares S. Gene expression profiles of early implant adherent cells in smokers and nonsmokers. *J Oral Implant*. 2015;41: 640–645.
18. Mutter GL, Zahrieh D, Liu C, et al. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics*. 2004;5:88.
19. Breer S, Hahn M, Kendoff D, et al. *Int Orthop*. 2012;36:2269–2274.
20. Hanzlik JA, Day JS. Bone ingrowth in well-fixed retrieved porous tantalum implants. *J Arthroplasty*. 2013;28:922–927.
21. Bobyn JD, Toh KK, Hacking SA, Tanzer M, Krygier JJ. Tissue response to porous tantalum acetabular cups: a canine model. *J Arthroplasty*. 1999;14: 347–354.
22. Bobyn J, Stackpool G, Hacking S, Tanzer M, Krygier J. Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *J Bone Joint Surg Br*. 1999;81:907–914.
23. Ivanovski S, Hamlet S, Salvi GE, et al. Transcriptional profiling of osseointegration in humans. *Clin Oral Implants Res*. 2011;22:373–381.
24. Mamalis AA, Silvestros SS. Analysis of osteoblastic gene expression in the early human mesenchymal cell response to a chemically modified implant surface: an in vitro study. *Clin Oral Implants Res*. 2011;22:530–537.
25. Kojima N, Ozawa S, Miyata Y, Hasegawa H, Tanaka Y, Ogawa T. High-throughput gene expression analysis in bone healing around titanium implants by DNA microarray. *Clin Oral Implants Res*. 2008;19:173–181.
26. Charoenpanich A, Wall ME, Tucker CJ, Andrews DM, Lalush DS, Loboa EG. Microarray analysis of human adipose-derived stem cells in three-dimensional collagen culture: osteogenesis inhibits bone morphogenic protein and Wnt signaling pathways, and cyclic tensile strain causes upregulation of proinflammatory cytokine regulators and angiogenic factors. *Tissue Eng Part A*. 2011;17:2615–2627.
27. Welldon KJ, Atkins GJ, Howie DW, Findlay DM. Primary human osteoblasts grow into porous tantalum and maintain an osteoblastic phenotype. *J Biomed Mater Res A*. 2008;84:691–701.
28. Liu H, Lin J, Roy K. Effect of 3D scaffold and dynamic culture condition on the global gene expression profile of mouse embryonic stem cells. *Biomat*. 2006;27:5978–5989.
29. Sagomonyants KB, Hakim-Zargar M, Jhaveri A, Aronow MS, Gronowicz G. Porous tantalum stimulates the proliferation and osteogenesis of osteoblasts from elderly female patients. *J Orthop Res*. 2011;29:609–616.
30. Smith JO, Sengers BG, Aarvold A, Tayton ER, Dunlop DG, Oreffo RO. Tantalum trabecular metal – addition of human skeletal cells to enhance bone implant interface strength and clinical application. *Tissue Eng Regen Med*. 2014;8:304–313.
31. Wang Q, Zhang H, Li Q, et al. Biocompatibility and osteogenic properties of porous tantalum. *Exp Ther Med*. 2015;9:780–786.
32. Nishimura I. Genetic networks in osseointegration. *J Dent Res*. 2013; 92(suppl 12):109s–118s.
33. Buchter A, Kleinheinz J, Meyer U, Joos U. Treatment of severe peri-implant bone loss using autogenous bone and a bioabsorbable polymer that delivered doxycycline (Atridox). *Br J Oral Maxillofac Surg*. 2004;42:454–456.
34. Klinge B, Meyle J. Peri-implant tissue destruction. The Third EAO Consensus Conference 2012. *Clin Oral Implants Res*. 2012;23(suppl 6):108–110.

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