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CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Genetic Networks in Osseointegration

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Abstract: *Osseointegration-based dental implants have become a well-accepted treatment modality for complete and partial edentulism. The success of this treatment largely depends on the stable integration and maintenance of implant fixtures in alveolar bone; however, the molecular and cellular mechanisms regulating this unique tissue reaction have not yet been fully uncovered. Radiographic and histologic observations suggest the sustained retention of peri-implant bone without an apparent susceptibility to catabolic bone remodeling; therefore, implant-induced bone formation continues to be intensively investigated. Increasing numbers of whole-genome transcriptome studies suggest complex molecular pathways that may play putative roles in osseointegration. This review highlights genetic networks related to bone quality, the transient chondrogenic phase, the vitamin D axis, and the peripheral circadian rhythm to elute the regulatory mechanisms underlying the establishment and maintenance of osseointegration.*

Key Words: dental implants, bone remodeling, gene expression, microarray analysis, cartilage matrix protein, circadian rhythm.

Introduction

Dental implant systems have become a viable treatment option among reconstructive strategies for partial and complete edentulous conditions (Das *et al.*, 2012; Pjetursson *et al.*, 2012). Dental implants comprise an endosseous anchoring component and a transmucosal abutment that supports various dental prostheses. The success of the dental implant depends on predictable biological responses to xenobiotic materials and incorporation of the endosseous implant fixture into the jawbone. The acquisition of a stable bone-implant relationship without clinical signs and symptoms of infection or inflammation is generally described as *osseointegration* (Branemark, 1983) and believed to play an integral role in the sustained immobility of the implant.

Rapid establishment of osseointegration is a goal of research and development to improve and broaden the clinical indications of dental implant systems. To date, many studies have reported numerous methods to modify the surface of the implant fixture (Iacono and Cochran, 2007). During prototyping, the biological effects of newly developed implant surfaces have been extensively characterized, in part according to the gene expression pattern of peri-implant tissues and cells. However, clinical outcome studies are lacking that translate these postulated effects to actual therapeutic benefits (Papaspyridakos *et al.*, 2012).

It must be noted that the molecular mechanism underlying osseointegration is not yet fully understood and specific genetic networks for targeted evaluation of successful osseointegration have not been identified. The objective of this review is not an exhaustive evaluation of a large volume of published articles; rather, it focuses on articles concerning genetic evaluations to identify molecular networks that are important to osseointegration.

Expression of Osteoblast Marker Genes

The bone interface of the endosseous component of titanium-based (Ti-based) implants does not exhibit the fibrous encapsulation reported for other types of implants; therefore, osseointegration has been considered to be synonymous to implant-induced bone formation. During prenatal and postnatal bone morphogenesis and development, uncommitted mesenchymal stem cells (MSCs) are subjected to a series of stimuli, such as bone morphogenetic protein–associated Smad phosphorylation and canonical wnt/β-catenin signaling that result in expression of the transcription factors Runx2 and osterix (Lian *et al.*, 2006; Sinha and Zhou, 2013). These "gatekeeper" transcription factors modify the pangenomic expression patterns of MSC and lead to ordered osteogenic differentiation. Fully differentiated osteoblasts are responsible for the synthesis of bone extracellular matrix (ECM), which is primarily composed of type I collagen (Col1) and noncollagenous molecules such as osteocalcin, osteopontin, osteonectin, and bone sialoprotein. It

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has been suggested that the embryonic molecular and cellular events associated with osteoblast differentiation and bone generation are recapitulated in the implant osteotomy site, which results in the establishment of osseointegration (Cooper, 1998; Moradian-Oldak *et al.*, 2006; Galli *et al.*, 2012).

This hypothesis has been addressed by examining the steady-state expression of osteoblast-related genes (Appendix Table). The reverse transcription polymerase chain reaction studies revealed that osteoblastic marker gene expression was observed in implant-adherent tissues of humans and experimental animals within several days after implant placement. The time course of osteoblastic marker gene expression in peri-implant tissues generally follows the temporal gene expression pattern during osteoblastic differentiation. However, the peak expression time points appeared to be influenced by implant type, material, and surface topography (additional description and references are provided in the Appendix Table). In addition, early expression of inflammatory cytokine-related genes has been noted (Omar *et al.*, 2010) and may play a role in the resolution of inflammation (Jinquan *et al.*, 2000) and fibrin remodeling (Jimbo *et al.*, 2007), thereby permitting the initiation of bone formation in the peri-implant zone.

Monjo *et al.* (2012) reported a positive correlation between the pullout force of Ti discs with a moderately rough surface and the area of adherent tissue as well as steady-state mRNA levels of osteoblast marker genes. Because the expression pattern of inflammatory cytokine genes did not show such correlation with the mechanical withstanding force of implant (Monjo *et al.*, 2012), peri-implant bone formation was considered critical to the establishment of osseointegration. However, in comparing the machined and moderately rough surface topography of implant fixtures, the expression profile of osteoblastic marker genes alone did not show a significant association with the mechanical withstanding force (Appendix Figure). During the prototyping of discrete deposition of hydroxyapatite nanoparticles onto a Ti surface via a chemically bonded silane molecular bridge, an unexpectedly robust increase in tolerable mechanical loading was observed 2 weeks after implant placement in rat femurs (Nishimura *et al.*, 2007). However, the bone volume normalized by tissue volume, as determined by 3-dimensional micro–computed tomography near the implant surface, was found to be relatively unchanged (Figure 1). Therefore, while bone formation around the implant may be a prerequisite for osseointegration, an exclusive focus on the simple osteoblast differentiation hypothesis will likely neglect other important genetic networks.

Molecular Network Regulating Bone Collagen Cross-Link

Several studies have demonstrated altered mechanical properties for the bone around implants (Butz *et al.*, 2006; Jimbo *et al.*, 2012; Saruwatari *et al.*, 2005; Takeuchi *et al.*, 2005). These studies used a nanoindentation assay to estimate intrinsic mechanical properties and generally found that bone tissue in close proximity to the implant surface became harder and stiffer than typical trabecular bone. In some reports, the hardness of the peri-implant bone was similar to that of cortical bone (Butz *et al.*, 2006).

Bone collagen fibers mainly comprise type I collagen, which undergoes a series of posttranslational modifications. In particular, collagen cross-linking occurs at the specific proline and lysine residues, hydroxylated by prolyl 4-hydroxylase (P4H), prolyl 3-hydroxylase (P3H), or lysyl hydroxylase. P3H forms a molecular complex with cartilage-associate protein (CRTAP) and cyclophilin E to conduct proline hydroxylation of collagen. Recently, recessive osteogenesis imperfecta patients with severe bone fragility phenotypes have been linked to deficiency of either P3H (Cabral *et al.*, 2007) or CRTAP (Barnes *et al.*, 2006). As such, the molecular network regulating collagen cross-link is considered to determine, in part, the bone mechanical properties.

Through a differential display polymerase chain reaction screening, P4H was identified as a gene that significantly upregulated with implant fixture placement (Ogawa and Nishimura, 2006). In a separate study, transient and early upregulation of CRTAP was identified in a whole-genome microarray analysis of human MSCs cultured on Ti discs (Wall *et al.*, 2009). Implant-induced upregulation of P4H or CRTAP may contribute to the increased collagen cross-linking leading to the increased hardness and stiffness of peri-implant bone tissue. Therefore, genetic networks that regulate posttranslational modifications of the bone collagen matrix may play an important role in determining mechanical properties of peri-implant bone.

Cartilage ECM in Peri-implant Tissue: Genomewide Transcriptome Analyses

Whole-genome microarray assays may be used to identify postulated implant-specific genetic networks (Table). The research strategy generally compares dynamically different steady-state levels of mRNA species among multiple experimental models. For example, transcriptome profiling of cell or tissue samples with or without implantation has been used to identify putative implant-induced gene networks (Carinci *et al.*, 2003; Kojima *et al.*, 2008), whereas studies comparing several time points have been used to investigate the sequence of events underlying osseointegration (Wall *et al.*, 2009; Donos *et al.*, 2011; Ivanovski *et al.*, 2011; Thalji *et al.*, 2013). Other studies have evaluated gene expression profiles associated with different implant surface treatments (Carinci *et al.*, 2004; Kim *et al.*, 2006; Sohn *et al.*, 2006; Mamalis and Silvestros, 2011; Ramis *et al.*, 2012).

The application of such strategies to *in vivo* studies surprisingly identified the expression of cartilage ECM genes such as hyaluronan and proteoglycan link protein 1 (HAPLN1) and type XI collagen (Col11a1) in peri-implant tissue. Other identified genes associated with chondrocyte differentiation included pannexin 3 (PANX3) and asporin (ASPN). Proteoglycans (PGs) were also identified by these studies (Table). Because wound

Figure 1.

Three-dimensional bone morphometry using micro–computed tomography. (**A**) Three-dimensional reconstruction of bone architecture around a titanium (Ti) implant integrated in rat femur. The implant image was digitally extracted. In each 3-dimensional data set, juxtaposing bone volume/tissue volume (BV/TV) was calculated at the cortical bone, the upper half (UH) of the trabecular bone, and the lower half (LH) of the trabecular bone. (**B**) The BV/TV value of the close proximity (24–48 μm) to the implant surface showed insignificant variations among different surface treatments: turned, turned with hydroxyapatite (HA) nanoparticle deposition, double acid etched (DAE; Osseotite, Biomet3I, Palm Beach Gardens, Florida), and DAE with HA nanoparticle deposition. Originally published in Nishimura *et al.* (2007). (**C**) Experimental implant fixture was placed in the osteotomy site of rat femur. After the establishment of osseointegration, bone tissue surrounding the implant fixture resisted the bone marrow remodeling. (**D**) A follow-up study found that the degree of osseointegration measured by the implant push-in test did not show any effect of HA nanoparticle coating on day 4 and week 1; however, there was the significant push-in value increase in the group of DAE/HA nanoparticle implants (black circles) over the DAE implant groups (gray circles). Originally published in Lin *et al.* (2009). The peri-implant bone volume (B) did not explain the increased degree of osseointegration at week 2.

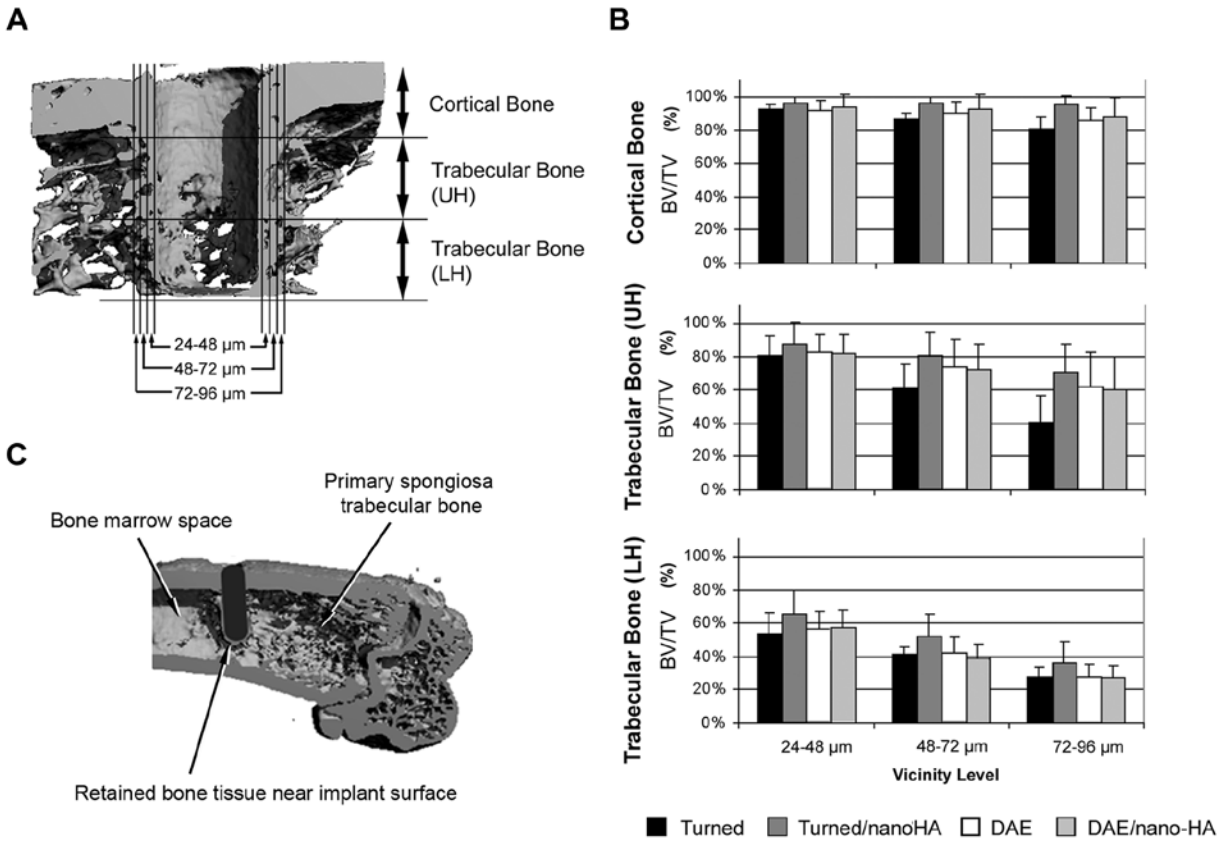
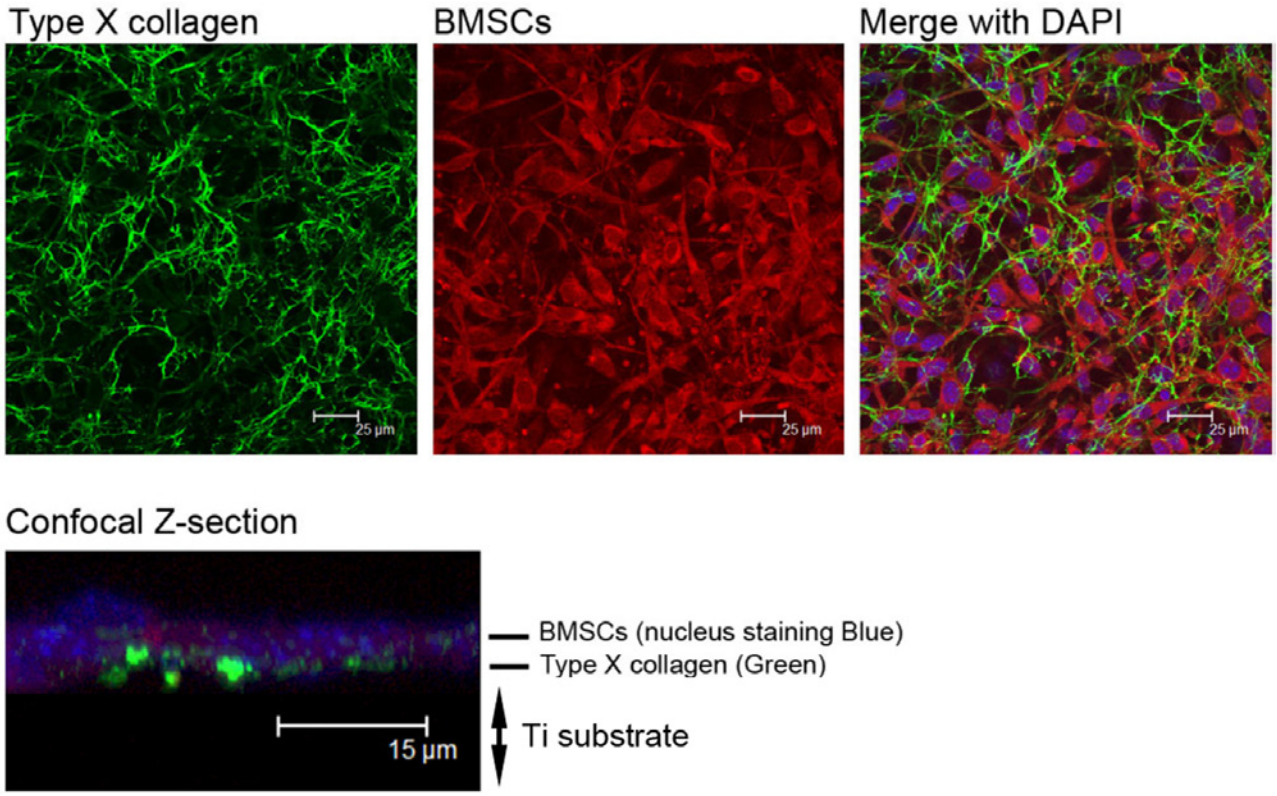


Table.
Whole-genome Transcriptome Microarray Studies on Peri-implant Tissues and Cells

Model	Implant	Microarray Platform	Experimental Strategy	Chondroblast-related Genes	Chondroblast-osteoblast-related Genes	Osteoblast-related Genes	References
In vivo							
Rat femur	Electrical discharge machined	Agilent Rat Oligo Array	Implant *vs.* osteotomy (weeks 1, 2, and 4)	Col11a1, HAPLN1,	BGN, FMOD, FN	MGP	Kojima *et al.*, 2008
Rat femur	Nanotite	Agilent Rat Whole Genome	Vitamin D deficient *vs.* control (week 2)	ACAN, Col2a1, Col9a2, Col9a3, Col10a1, Col11a1, DSPG3, HAPLN1	—	—	Mengatto *et al.*, 2011
Rat tibia	AT-1, AT-2	Affymetrix Rat Gene 1.1 ST	Day 2 *vs.* day 4	PANX3, ASPN, Col11a1	DCN, DMP1	OCN, OMD	Thalji *et al.*, 2013
Human mandible	SLActive	Illumina Human WG-6 V3	Day 4 *vs.* day 7; day 7 *vs.* day 14; day 4 *vs.* day 14	CHAD, Col11a1, Col11a2	—	OCN	Ivanovski *et al.*, 2011
Human mandible	SLA, SLActive	Illumina Human WG-6 V3	SLA *vs.* SLActive (days 4, 7, and 14)	Reported genes were nonspecific to tissue type			Donos *et al.*, 2011
In vitro							
MG63 cells	TPSS	Human 19.2K (Ontario Cancer Institute)	Plastic dish *vs.* TPSS disk	Reported genes were nonspecific to tissue type			Carinci *et al.*, 2003
MG63 cells	NanoPORE, Machined	Human 19.2K (Ontario Cancer Institute)	NanoPORE *vs.* Machined	Reported genes were nonspecific to tissue type			Carinci *et al.*, 2004
MG63 cells	S, SLA, HA, HF, TIN, DLC	15K human verified sequences master set	Hierarchical clusters	Reported genes were nonspecific to tissue type			Kim *et al.*, 2006
MG63 cells	Anodized, HA coating with different thicknesses	15K human verified sequences master set	ANOVA	Reported genes were nonspecific to tissue type			Sohn *et al.*, 2006
hMSCs	SLA, SLActive	Affymetrix Human Genome U133 Puls 2.0	3 h *vs.* 24; 3 h *vs.* 72 h; 3 h *vs.* 120 h	ACAN, CILP, CRTAP, HAPLN1, HAPLN3, TNS1	FN	OPN	Wall *et al.*, 2009
hMSCs	Relatively smooth (SMO), SLA, SLActive	cDNA GEArray for Human Osteogenesis	SLA *vs.* SMO; SLActive *vs.* SMO	SOX9	BMP6, BMPR1A, ICAM1, SMAD5, SMAD6, SMAD7, SMAD9	Col5A1, ON, OPN	Mamalis and Silvestros, 2011
NHOst cells	Polished, Polished + HF, Grid blasted, Grid blasted + HF	Affymetrix Human Genome U133 Puls 2.0	ANOVA	Discussions and reports focusing on osteoblast-related genes			Ramis *et al.*, 2012

Cartilage-related genes: ASPN, aspirin; ACAN, aggrecan; Col2a1, α1(II) collagen; CHAD, chondroadherin; CILP, cartilage intermediate layer protein; Col9a2, α2(IX) collagen; Col9a3, α3(IX) collagen; Col10a1, α1(X) collagen; Col11a1, α1(XI) collagen; CRTAP, cartilage associated protein; DSPG3, dermatan sulfate proteoglycan 3; HAPLN1, hyaluronan and proteoglycan link protein 1; HAPLN3, hyaluronan and proteoglycan link protein 3; PANX3, pannexin 3; SOX9, SRY-box 9; TNS1, tensin 1.
Cartilage or bone-related genes: BGN, biglycan; BMP6, bone morphogenetic protein 6; BMPR1A, BMP receptor 1A; DCN, decorin; DMP1, dentin matrix acidic phosphoprotein 1; FMOD, fibromodulin; FN, fibronectin; ICAM1, intercellular adhesion molecule 1; SMAD5/SMAD9, receptor-regulated SMADs; SMAD6/SMAD7, inhibitor SMADs.
Bone-related genes: Col5A1, α1(V) collagen; MGP, matrix gla protein; OCN, osteocalcin; OMD, osteomodulin; ON, osteonectin; OPN, osteopontin.

Figure 2.
Mouse bone marrow stem cells (BMSC; D1 ORL UVA [D1]) were cultured on acid-etched titanium (Ti) discs with discrete calcium phosphate nanoparticle deposition (Nanotite, Biomet3I, Palm Beach Gardens, Florida). The D1 cell culture was stained for Col10a1 (green), F-actin (red), and nuclei (DAPI). Confocal laser-scanning micrographs indicated the presence of Col10a1 in the interface zone between BMSC and Ti disc (unpublished, F. Mussano and I. Nishimura).



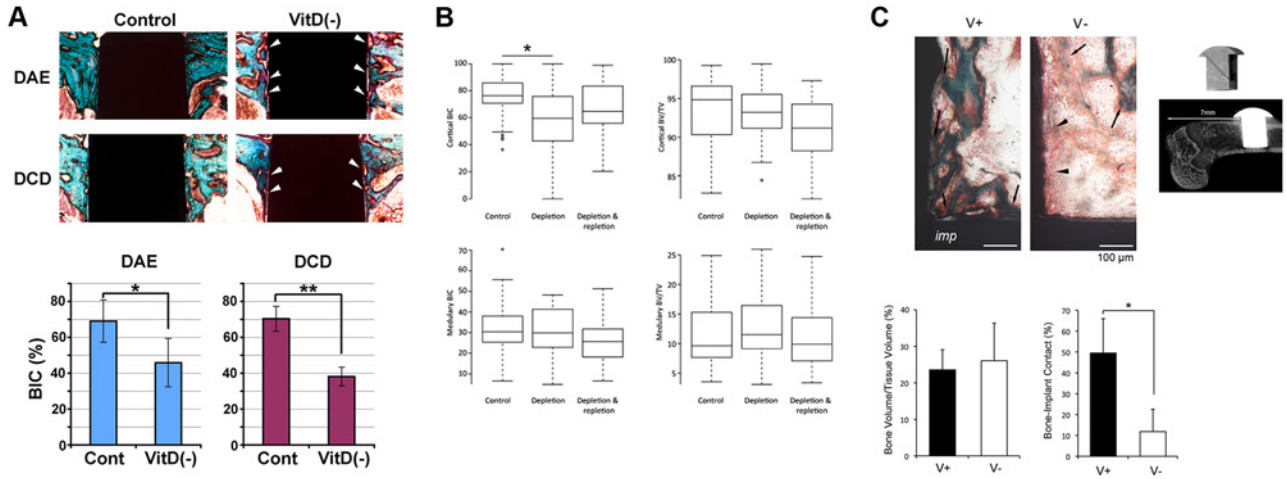
healing after implant surgery involves the regeneration of intramembranous bone, which is devoid of cartilage callus, the expression of cartilage-related genes was unexpected. *In vitro* studies using human MSCs cultured on implant discs similarly demonstrated early upregulation of HAPLNs, CRTAP, and SOX9; however, the MG63 human osteosarcoma cell line did not differentially express cartilage-related genes when cultured on implant discs (Table).

Transient expression of a chondrogenic phenotype has been reported during intramembranous ossification in the embryonic development of calvaria (Nah *et al.*, 2000) and during wound healing after tooth extraction (Ting *et al.*, 1993; Devlin *et al.*, 1995; Ting *et al.*, 1999). In these models, clusters of condensed MSCs produced cartilage-related ECM that was found to be highly localized at the osteogenic front rather than within the bone tissue. Furthermore, a loss-of-function mutation of a cartilage-related ECM molecule, Col9a1, was shown to decrease the formation of woven trabecular bone in the extraction socket (Ting *et al.*, 1999). Therefore, the transient expression of cartilage-related ECM molecules may be part of an early bone generation or regeneration process, and the cartilaginous ECM produced may serve as a template or interface layer to guide subsequent bone apposition.

The interface between the implant surface and bone consists of an amorphous layer containing PG and a layer with randomly aligned collagen filaments (Albrektsson *et al.*, 1983), although the amount of PG in the interface zone has been debated (Klinger *et al.*, 1998; Palmquist *et al.*, 2010). PG is functionally modified with glycosaminoglycan side chains, and it has been reported that the adhesion of *in vitro* mineralized tissue to Ti discs was attenuated by treatment with chondroitinase AC, chondroitinase B, or keratinase (Nakamura *et al.*, 2006), which suggests a functional role of glycosaminoglycans/PGs in bone adhesion to the implant surface.

Furthermore, new genetic network data suggest that the peri-implant tissue may also contain various cartilage-related ECM molecules, such as type X collagen (Figure 2). Type X collagen is a member of the "network forming" collagens and has been identified in the underlying calcified zone of articular cartilage and growth plate. Notably, in weight-bearing limbs, the prominent localization of type X collagen with PGs was limited to the longitudinal septa of primary spongiosa undergoing endochondral ossification (Gibson *et al.*, 1996). Type X collagen was also found in noncartilage tissues, such as mineralized interface zone between bone and ligament (Niyibizi *et al.*, 1996) and vertebral bone

Figure 3.
The decreased bone-to-implant contact (BIC) in vitamin D–deficient rats. (**A**) Cylinder-shape implant (1-mm diameter and 2 mm long) with double acid-etched surface (DAE; Osseotite, Biomet3I, Palm Beach Gardens, Florida) or DAE surface and discrete calcium phosphate nanoparticle deposition (DCD; Nanotite, Biomet3I) were placed in the femur of control and vitamin D–deficient, "VitD(–)," rats. After 2 weeks of healing, BIC measured in nondecalcified histologic sections was significantly decreased in VitD(–) rats. $^{*}p < .05$, $^{**}p < .01$. Originally published in Kelly *et al.* (2009b). (**B**) The reduced BIC at the transcortical bone of tibia was demonstrated in ovariectomized and vitamin D deficient rats. This effect was attenuated by 2400 IU/kg of vitamin D supplementation. $^{*}p < .05$. From Dvorak *et al.* (2012) with permission. (**C**) T-shaped titanium implant with DCD surface (Nanotite, Biomet3I) was placed in the femur of control (V+) and vitamin D–deficient (V–) rats. After 2 weeks, BIC measured in the hollow chamber was significantly reduced in the V– group, whereas the total bone area was not affected. $^{*}p < .05$. Originally published in Mengatto *et al.* (2011).



and intervertebral disc (Roberts *et al.*, 1998). From the biomechanical requirement of these interface tissues, it has been postulated that type X collagen may supply tissues with considerable tensile strength. The presence of type X collagen at the interface zone between Ti implant and peri-implant tissue is a novel but preliminary observation (Figure 2). It is tempting to speculate that type X collagen might provide a critical biomechanical advantage of osseointegrated implant.

Vitamin D Axis and Circadian Rhythm Genetic Networks

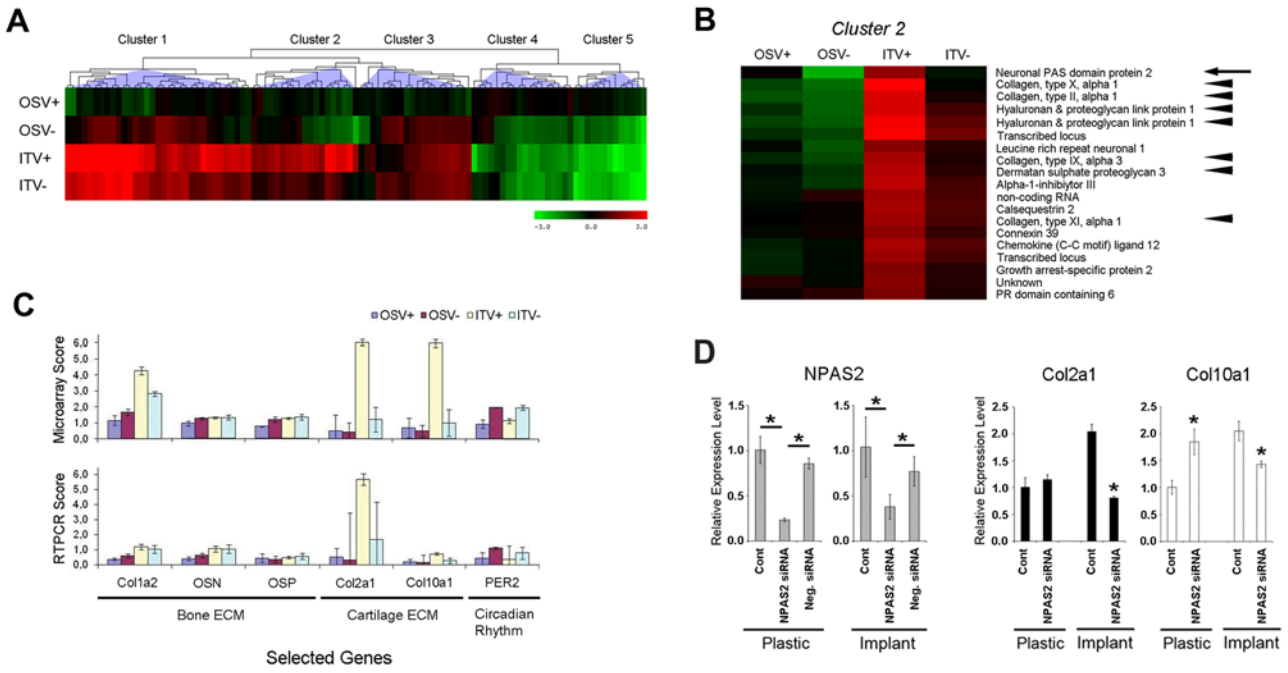
Osteogenic differentiation of MG63 cells cultured on moderately rough Ti discs was found to be accelerated by 1,25-dihydroxyvitamin D3 supplementation (Lossdorfer *et al.*, 2004), and this effect was further enhanced by lysophosphatidic acid (Mansell *et al.*, 2010). Experimental animal studies have demonstrated decreased osseointegration in diet-induced vitamin D deficiency (Kelly *et al.*, 2009a; Mengatto *et al.*, 2011; Dvorak *et al.*, 2012). Vitamin D deficiency decreased the bone-to-implant contact but did not affect the overall bone volume around the experimental implant (Figure 3). Therefore, it is likely that vitamin D metabolites influence the mechanism of osseointegration.

1,25-dihydroxyvitamin D3 is an active form of vitamin D that binds to the vitamin D receptor, which then forms molecular complexes with other transcription factor partners to regulate the expression of a wide range of target genes. We have applied the whole-genome microarray technique to elucidate the effect of vitamin D deficiency on implant osseointegration (Mengatto *et al.*, 2011). Four independent peri-implant tissue samples from osteotomy sites with or without implant placement in rats sufficient or deficient in vitamin D were compared in this study. Hierarchical cluster analysis with the bootstrap correction revealed that implant placement was the most significant factor affecting gene expression (Figure 4A). Among 103 genes identified by 2-way analysis of variance, the most significantly affected genes were neuronal PAS domain protein 2 (NPAS2) and period homolog 2 (Per2), which are involved in the circadian rhythm. The hierarchical cluster affected most by vitamin D deficiency included NPAS2. To our surprise, this cluster further comprised several cartilage-related ECM genes, such as HAPLN1, Col2, Col9, Col10, and Col11 (Figure 4B). The expression of these cartilage-related ECM genes was robustly influenced by implant placement and vitamin D level, which did not modulate the expression of bone-related ECM genes (Figure 4C). Knockdown of NPAS2 by siRNA affected the steady-state mRNA levels of Col2 and Col10 (Figure 4D), which suggests that NPAS2 may play a role in the transcriptional regulation of cartilage-related ECM genes in peri-implant tissue.

Proposed Model of Osseointegration Genetic Networks

While Ti material has been considered bioinert, the presence of a Ti implant

Figure 4.
Whole-genome microarray analysis of the effect of vitamin D deficiency on differential transcriptome in peri-implant tissue. Control (vitamin D sufficient, V+) and vitamin D–deficient (V–) rats received osteotomy surgery in the femur, which was either left to heal (OS) or filled with T-cell titanium implant (IT) with discrete calcium phosphate nanoparticle deposition surface (Nanotite, Biomet3I, Palm Beach Gardens, Florida). Peri-implant tissue was collected from the hollow chamber of the T-cell implant and subjected to microarray analyses. (**A**) Four independent animals in each group were used for 2-way analysis of variance and hyachical cluster analysis. The presence of implant was the most influencing factor for differential transcriptome expression. (**B**) Cluster 2 suggested the most significant modulation between ITV+ and ITV–, which included neuronal PAS domain protein 2 (NPAS2; arrow) and a number of cartilage ECM genes (arrowheads). (**C**) Microarray data were confirmed by reverse transcription polymerase chain reaction that vitamin D deficiency and/or the placement of implant modulated cartilage ECM genes while minimal effects were found in bone ECM genes. (**D**) NPAS2 knockdown by siRNA in D1 cells that were cultured on plastic culture dish or titanium disc affected the expression of cartilage ECM Col2a1 and Col10a1. Originally published in Mengatto *et al.* (2011).

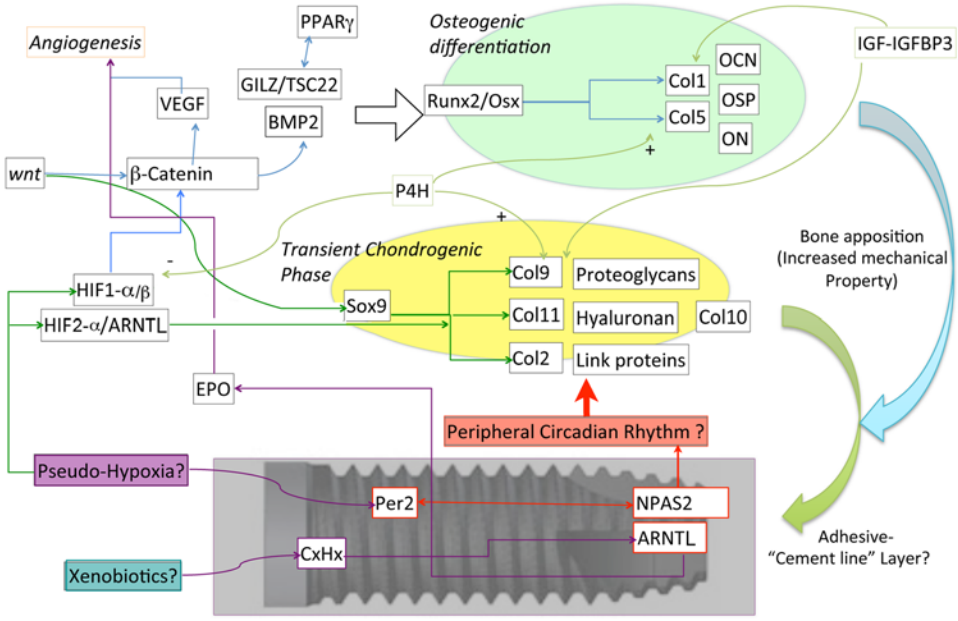


fixture appears to be a significant factor modulating gene networks in peri-implant tissue. Two potential models may be proposed to regulate unique genetic networks leading to the establishment of osseointegration (Figure 5).

During manufacturing, the Ti implant surface is subjected to oxidization to form a thin layer of TiO_2. Because the implant surface is hardly saturated by TiO_2, it is possible that the Ti implant continues to be oxidized after surgical placement in the bone marrow. If so, the oxygen level of the microenvironment near the Ti surface may be depleted, thus potentially creating a hypoxic zone. It is well established that a hypoxic environment can induce chondrogenic differentiation as indicated by the expression of the chondrogenic transcription factor Sox9 and cartilage-related ECM molecules (Kanichai *et al.*, 2008; Adesida *et al.*, 2012). Although this mechanism is speculative, BMSCs may thus undergo chondrogenic differentiation in the postulated hypoxic microenvironment at the Ti implant surface (Figure 5).

Although the hyperallergic immune response is extremely rare in humans, the other mechanism involves biological response to implant fixture that must be recognized as a foreign body by resident cells. One responding mechanism utilizes a sensor and signaling mechanism involving a group of proteins with per-ARNT-sim (PAS) transcriptional DNA-binding domains (Moglich *et al.*, 2009). PAS sensors respond to hypoxia and toxic environments (Taylor and Zhulin, 1999) invoking a cascade of cellular responses. Interestingly, molecules involved in the circadian rhythm also possess PAS domain. In addition to the central circadian rhythm expressed in the suprachiasmatic nucleus, peripheral tissues have their own circadian clock mechanism. Microarray analysis of mouse calvaria harvested every 4 hours revealed that nearly 30% of genes demonstrated circadian oscillation (Zvonic *et al.*, 2007). In the maxilla-mandibular complex, the expression of osteocalcin demonstrated circadian oscillation (Gafni *et al.*, 2009). Although the role of peripheral bone circadian rhythm has not been explored, the new observation that implant-induced microenvironment appeared to have a significant impact on the regulation or dysregulation of NPAS2 and Per2 expression in peri-implant tissue (Mengatto *et al.*, 2011) may provide a novel clue to understand the mechanism of osseointegration (Figure 5).

Figure 5.
Proposed genetic networks of osseointegration. The osteotomy procedure used to prepare an implant placement site creates an ablation wound in the bone marrow. Intramembranous ossification occurs during the healing of bone marrow ablation without cartilage tissue formation. Mesenchymal stem cells (MSCs) in bone marrow possess multipotent differentiation capability and undergo osteoblastic differentiation with a sufficient oxygen supply. Under hypoxic conditions, however, MSCs can develop chondrogenic differentiation. The puzzling expression of a mixture of osteoblastic and chondroblastic genes in the peri-implant tissue may be induced by the microenvironment derived from titanium (Ti) implant fixture. The proposed mechanisms may involve a thin layer of hypoxic zone due to continuous oxidization of Ti implant fixture, affecting the differentiation course of MSCs. It is also possible that MSCs may recognize implant fixture as a foreign material and activate xenobiotic responses. Either a hypoxic or xenobiotic microenvironment may commonly stimulate PAS domain-containing molecular sensors. ARNTL (aryl hydrocarbon receptor nuclear translocator-like) is such a PAS sensor for hypoxic or xenobiotic cell response. ARNTL has been found to dimerize with hypoxia-inducible factors and thus potentially activates the hypoxia-inducible factor/ARNTL pathway for chondrogenic differentiation, at least in a transient phase. Separately, ARNTL is also an essential circadian rhythm molecule. ARNTL dimerizes with NPAS2 or CLOCK and regulates the transcription of Per and Cry molecular clock proteins. Therefore, the implant fixture-mediated microenvironment may have a significant effect on the peripheral circadian rhythm. Furthermore, NPAS2, one of the most affected genes by the implant placement and vitamin D deficiency, was found to directly upregulate Col2 and Col10 in mouse MSCs (Mengatto *et al.*, 2011). It may be suggested that an alternative pathway may regulate the expression of a selected set of cartilage ECM molecules without full chondrogenic differentiation. Small but critical synthesis of proteoglycans has been postulated to facilitate the adhesion of bone tissue to implant surface. In addition, although highly speculative, the peri-implant bone may contain a mixture of bone and cartilage ECM. Type X collagen may increase the tensile strength of interface tissues such as peri-implant bone. In addition, a study on Col9 knockout bone showed significantly increased susceptibility to osteoclastic bone resorption (Wang *et al.*, 2008). The long-term resistance of peri-implant bone against catabolic remodeling might be contributed, in part, by its unique ECM composition.



Conclusions and Future Perspectives

The term *osseointegration* was first introduced as an interpretation of histologic observations that bone and bone marrow tissues exhibited a close contact with the surface of the endosseous component of Ti-based implants without noticeable formation of fibrous tissues (Branemark *et al.*, 1977). The pattern of bone formation on the implant surface has subsequently been intensively characterized in cell culture systems and animal models as well as biopsy tissue samples from humans. However, these investigations have not elucidated the mechanism underlying osseointegration. Recent transcriptome data have indicated that unexpectedly complex genetic networks are associated with implant osseointegration, some of which were summarized in this review.

The major advantage of current implant systems is long-term stability, which is largely contributed by sustained maintenance of osseointegration. In fact, the peri-implant bone exhibits unique characteristics, including the strong adhesion to implant fixture and the unusual resistance to catabolic remodeling. Therefore, increasing reports on the loss of osseointegration after years of service may not be addressed by the simple

infection/inflammation model alone. In future studies, the validity and significance of the postulated genetic networks must be carefully established, which should provide the basis for the acquisition and maintenance of osseointegration.

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