Gene Expression Profiles of Early Implant Adherent Cells in Smokers and Nonsmokers

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The objective of this study was to evaluate the impact of smoking on the early molecular events involved in peri-implant healing at either a micro-roughened or a micro-roughened with superimposed nanofeatures surface implant in humans. Twenty-one subjects, 10 smokers and 11 nonsmokers received 4 mini-implants (2.2 × 5.0 mm; 2 of each surface). After 3 and 7 days, paired mini-implants were retrieved by reverse threading and RNA isolated from implant adherent cells. Whole genome microarrays were used interrogate the gene expression profiles. The study failed to identify differences in the gene expression profiles of implant adherent cells at this early stage of osseointegration (up to day 7) comparing smoker and nonsmoker individuals.

Key Words: smoking, dental implants, osseointegration, microarray, gene expression

INTRODUCTION

Smoking detrimentally influences wound and bone fracture healing.[1,2] Early clinical studies indicated that smoking had deleterious effects on dental implant integration represented by either early or late implant failures.[3–7] In a recent systematic meta-analysis Strietzel and coworkers reported a significantly enhanced risk for implant failures among smokers (implant-related OR 2.25, $CI_{95\%}$ 1.96–2.59; patient related OR 2.64; $CI_{95\%}$ 1.70–4.09) compared with nonsmokers.[4] However, this analysis did not segregate the effects of implant surface topography or timing on such failures. Further, based on results of 5 studies,[8–12] no significant impact on prognosis of implants with particle-blasted, acid etched, or anodic oxidized surfaces were noted. While early failures are attributed to the inability of the host to establish an intimate bone-implant contact following implant placement, late failure are often associated with peri-implantitis, plaque related gingivitis, and or occlusal overloading.[13]

Efforts to comprehend the impact of tobacco smoke/nicotine on early implant fixation using in vivo animal models elucidated that intermittent cigarette smoke inhalation may result in lower bone-to-implant contact and less bone area within the threads.[14–19] Similarly, a prospective histomorphometric study in humans demonstrated a significantly reduced bone-to-implant contact at 8 weeks (early bone healing) in smoking patients.[20] Recent data demonstrated that, compared with machined cp titanium surfaces, surfaces with increased surface roughness (in particular moderately roughened surfaces (S [a] values 1–2 μm) improved bone-to-implant contact and provided higher resistance to torque removal.[21,22] In a histometric analysis of implants retrieved from type IV bone (8 weeks after implant placement) of smoking patients, D'Avila and colleagues demonstrated a higher bone-to-implant contact adjacent to implants with moderately roughened surfaces (sandblasted acid etched) compared to machined implants.[23] The superimposition of nanoscale features on microroughened surfaces augmented bone deposition, which was attributed to enhancement of proliferation, differentiation, and increased expression of osteogenic markers.[24–27] Nanoscale features are topographies less than 100 nm in at least 1 dimension, which may include several forms such as nanostructures, nanocrystals, nanocoatings, nanoparticles, and nanofibers.[28]

The mechanisms by which smoking has been proposed to impair wound healing include:[29] (1) reduced oxygenation of healing tissues due to the higher affinity of carbon monoxide for hemoglobin, nicotine vasoconstrictive effects,[30,31] increased platelet aggregation and adhesiveness, and (2) cytotoxic effects on fibroblasts and inflammatory cells, particularly polymorphonuclear cells.[32,33] Yet, the molecular and cellular mechanisms defining the impact of smoking on early osseointegration remains poorly defined. Further, how implant surface modification affects early gene expression in smokers remains largely unexplored.

The ongoing accumulation of knowledge of the early molecular events of osseointegration indicate a wide array of cell types involved in the establishment of the implant bone interface[34] (Thalji et al, 2013). To date, the influence of systemic and habitual factors on these various molecular aspects of osseointegration have not been described. The aim of this study was to investigate the effects of smoking on the gene expression profiles of early implant adhering cells on microroughened and microroughened implants with superimposed nanofeatures.

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Materials and Methods

Subjects

The study protocol was approved by the Institutional Review Board (IRB) at University of North Carolina at Chapel Hill (IRB protocol #10-1963).

The study enrolled a total of 21 partially or completely edentulous systemically healthy subjects. Of these, 11 were nonsmoking individuals with a mean age of 60.2 years (range, 47 to 69 years) and 10 were smokers with a mean age of 50.8 years (range, 33 to 63 years). Enrollment criteria included the absence of at least 2 teeth, with adequate bone volume to allow placement of 4 mini-implants without impingement on vital anatomical structures (eg, maxillary sinus, mandibular nerve, adjacent teeth) and an edentulous period of at least 6 months. Smokers were included based on self reported smoking habit of >10 cigarettes a day. Exclusion criteria included pregnancy, a diagnosis of any systemic condition that could affect bone healing such as uncontrolled diabetes, and a history of radiotherapy in the head and neck region. Individuals taking corticosteroids or bisphosphonates, and so forth, were also excluded.

Mini-implants and surface preparation

Eighty-four screw-shaped mini-implants (2.2×5.0 mm) made of grade IV commercially pure titanium were used in this study (Dentsply AstraTech, Mölndal, Sweden). The mini-implants were manufactured to the same specifications as commercially available implants. Briefly, the mini-implants were prepared with 2 surface topographies. One group was blasted with TiO_2 (75μm particles) (AB TiOBlast, Dentsply AstraTech) creating a moderately roughened surface, while the other group was blasted with TiO_2 particles and then treated with hydrofluoric acid (HF) immersion protocol according to Osseospeed manufacturing procedure (AB Osseospeed, Dentsply AstraTech) creating a moderately roughened surface with super-imposed nanofeatures.[27] All mini-implants were washed with sterile water and beta-sterilized according to standard protocols for manufacture of dental implants. Beta-sterilization is a method of sterilization that is dependent on beta-particles, free electrons, which are transmitted through a high-voltage electron beam from a linear accelerator. As these high-energy free electrons penetrate into matter, they produce their effect by ionizing the atoms they hit, producing secondary electrons that kill the microorganisms through disruption of the DNA molecule, therefore preventing cellular division and propagation of biologic life.

Surgery

Following local anesthesia, 2 separate crestal incisions approximately 6 mm in length were performed and full thickness mucoperiosteal flaps elevated. Each subject received 4 implants (2/surface). Two separate surgical sites were identified and full osteotomies were prepared using 2.0 mm single use twist drills under profuse saline irrigation to allow placement of 2 mini-implants per surgical site (1 TiOblast [TiO] and 1 Osseospeed [OS]). This paired surgical design permitted the retrieval of 2 mini-implants (1 of each surface) on day 3 without disturbing the healing of the other 2 implants on day 7. Implants were placed manually and primary stability was achieved at the time of implant placement. A submerged approach was used and the flaps were approximated and closed primarily with 4–0 chromic gut sutures.

Implant retrieval

At 3 and 7 days following surgery, 1 surgical site was chosen at random, re-entered and the paired (TiO, OS) implants removed by reverse threading. The implants were immediately rinsed in ice-cold PBS (phosphate-buffered saline), placed into sterile 1.5-mL centrifuge tubes containing Tri-reagent (Invitrogen, Carlsbad, Calif) and vortexed vigorously. Cell lysates were snap frozen, and stored at $-80^{\circ}C$ until further use. Tissues were reapproximated and closed with 4.0 chromic gut sutures.

RNA isolation and microarray hybridization

Total RNA was isolated using the standard Tri-reagent protocol and collected by ethanol precipitation followed by a purification step using RNeasy MinElute Clean up kit (Qiagen, Valencia, Calif) according to manufacturer's recommendations. RNA was assessed for quality and quantity using the Bioanalyzer (Agilent, Santa Clara, Calif) and Nanodrop ND-1000 spectrophotometer (Nanodrop, Wilmington, Del), respectively. Samples were labeled, processed, and hybridized to the Affymetrix Human Gene 1.1 ST Array at the UNC genomic core facility following the manufacturer's recommended protocol and reagents (Affymetrix, Santa Clara, Calif). The Human Gene 1.1 ST Array Plate interrogates more than 27,000 well-annotated genes. The raw microarray data is available online at the NCBI-GEO (http://www.ncbi.nlm.nih.gov/geo/) database, accession number GSE42288.

Microarray data analysis

Data analysis was completed using the GeneSpring software v. 12.0 (Agilent). For statistical analysis 3-way ANOVAs were applied to determine differentially expressed genes among the various parameters (smoking, implant surface type, and time points). Further analysis included pairwise comparisons of each implant surface independently at the different time points for each group of patients (day 7 vs day 3). A *P*-value of 0.05 was set as the threshold for statistical significance. Exported gene lists included significant genes; fold changes and *P*-values for comparisons. These lists of genes were then condensed into organized classes of related biology using the Gene Ontology Consortium (GO) Ontology Browser (http://www.geneontology.org/) function in GeneSpring. The Hochberg false discovery rate method [35] was applied to correct for multiple sampling.

Results

Microarray

A total of 84 mini-implants with either microroughened or nanoroughened surface topography were placed in 21 smoking and nonsmoking subjects. without reported adverse reactions. Adherent cells on retrieved implants provided ample intact RNA

TABLE 1

The top 35 differentially expressed genes (*P*-value $\leq$ 0.05) at Osseospeed (OS) (day 7 vs day 3) in the nonsmoker group with their fold regulation in OS, TiOblast (TiO) in both subjects' groups

Transcript Cluster ID	Gene Symbol	Gene Description	FC ([Healthy-OS-Day 7] vs [Healthy-OS-Day 3])	FC ([Smokers-OS-Day 7] vs [Smokers-OS-Day 3])	FC ([Healthy-TiO-Day 7] vs [Healthy-TiO-Day 3])	FC ([Smokers-TiO-Day 7] vs [Smokers-TiO-Day 3])
7951297	MMP12	Matrix metallopeptidase 12 (macrophage elastase)	9.66	9.53	12.78	10.29
7919815	CTSK	Cathepsin K	9.13	7.89	9.71	8.33
8093104	TM4SF19	Transmembrane 4 L six family member 19	9.02	8.64	11.80	10.85
7951217	MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)	7.67	7.19	10.45	7.77
8063115	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	7.66	5.92	9.85	7.71
7965403	LUM	Lumican	5.87	9.43	6.22	6.44
7923547	CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	5.58	6.01	9.62	8.09
8134263	COL1A2	Collagen, type I, alpha 2	5.37	7.92	5.45	6.70
8046922	COL3A1	Collagen, type III, alpha 1	5.29	8.39	5.25	6.30
8059905	COL6A3	Collagen, type VI, alpha 3	5.12	7.74	5.28	6.30
8016646	COL1A1	Collagen, type I, alpha 1	5.03	6.77	4.92	6.31
7923562	CHIT1	Chitinase 1 (chitotriosidase)	4.96	5.80	7.97	8.12
7971077	POSTN	Periostin, osteoblast specific factor	4.94	6.97	4.38	4.71
7965410	DCN	Decorin	4.47	7.43	4.38	4.48
8034304	ACP5	Acid phosphatase 5, tartrate resistant	4.19	3.68	4.68	3.66
8109344	GM2A	GM2 ganglioside activator	4.10	3.60	5.12	4.24
8127563	COL12A1	Collagen, type XII, alpha 1	3.94	5.58	3.48	3.50
8115327	SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	3.84	5.70	4.52	5.22
8161044	TPM2	Tropomyosin 2 (beta)	3.76	4.39	3.15	3.31
8170648	BGN	Biglycan	3.67	5.53	4.38	5.00
8144917	LPL	Lipoprotein lipase	3.49	3.48	3.76	3.46
7898805	C1QB	Complement component 1, q subcomponent, B chain	3.45	4.34	3.84	3.70
8069269	COL6A1	Collagen, type VI, alpha 1	3.34	4.39	3.28	3.79
8149448	MSR1	Macrophage scavenger receptor 1	3.32	2.67	3.00	2.63
7942064	GAL	Galanin prepropeptide	3.31	3.77	7.26	5.01
8075635	TIMP3	TIMP metallopeptidase inhibitor 3	3.29	3.25	3.18	3.27
8131844	GPNMB	Glycoprotein (transmembrane) nmb	3.27	3.08	4.04	3.13
8175531	CDR1 | YTHDC2	Cerebellar degeneration-related protein 1, 34kDa | YTH domain containing 2	3.22	4.05	3.03	3.59
8151532	FABP4	Fatty acid binding protein 4, adipocyte	3.16	1.67	1.67	2.11
7934920	LIPA	Lipase A, lysosomal acid, cholesterol esterase	3.12	2.91	3.26	2.80
7898799	C1QC	Complement component 1, q subcomponent, C chain	3.06	3.51	2.83	3.12
8124388	HIST1H3B	Histone cluster 1, H3b	3.05	2.80	3.06	3.00
7951309	MMP13	Matrix metallopeptidase 13 (collagenase 3)	3.04	2.84	2.59	1.97
8006594	CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	3.03	3.16	2.68	2.05
7907160	ATP1B1 | NME7	ATPase, Na+/K+ transporting, beta 1 polypeptide | non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase)	3.03	2.58	2.72	3.01

for analysis. A 3-way ANOVA was applied to determine differentially expressed genes among the various parameters (smoking status, implant surface type, and time points). No genes were identified as differentially expressed (*P*-value $<$ 0.05) when comparing the effects of smoking on either implanted implant surface. Importantly, when the variable of time was evaluated, statistically significant expression of genes was identified. Analysis of time-course dependent (day 7 vs day 3) gene expression in implant-adherent cells for each test surface (OS, TiO) in smokers and nonsmokers was performed to interrogate the molecular events occurring at the very early stages of osseointegration. The top 35 differentially expressed genes (*P*-value $\leq$ 0.05) in the OS, nonsmoking group (day 7 vs day 3) with their fold regulation in OS, TiO in both subjects' groups are listed in Table 1. Table 2 includes the top 25 differentially downregulated genes in the OS (day 7 vs day 3), nonsmoking group with their fold regulation in OS, TiO in both subjects' groups. At this early time point similar trends in gene expression were noted in implant-adherent cells regardless of implant surface and smoking status.

Identification of gene ontologies associated with smoking status and implant surface

GO analysis identified the functional categories overrepresented in the gene lists of the differentially expressed genes between day 7 and day 3 (fold change $\geq$ 2; *P*-value $\leq$ 0.05) independently for each subjects' group at both implant

TABLE 2

The top 25 differentially downregulated genes at Osseospeed (OS) (day 7 vs day 3) in the nonsmoker group with their fold regulation in OS, TiOblast (TiO) in both subjects' groups.

Transcript Cluster ID	Gene Symbol	Gene Description	FC ([Smokers-OS-Day 7] vs [Smokers-OS-Day 3])	FC ([Healthy-OS-Day 7] vs [Healthy-OS-Day 3])	FC ([Healthy-TiO-Day 7] vs [Healthy-TiO-Day 3])	FC ([Smokers-TiO-Day 7] vs [Smokers-TiO-Day 3])
8149116	DEFA3|DEFA1|DEFA1B	Defensin, alpha 3, neutrophil-specific | defensin, alpha 1 | defensin, alpha 1B	−2.65	−3.20	−2.67	−3.38
8149137	DEFA3|DEFA1|DEFA1B	Defensin, alpha 3, neutrophil-specific | defensin, alpha 1 | defensin, alpha 1B	−2.65	−3.20	−2.67	−3.38
7920205	SPRR2A|SPRR2B	Small proline-rich protein 2A | small proline-rich protein 2B	−1.95	−2.89	−2.95	−1.08
7920214	SPRR2E	Small proline-rich protein 2E	−2.09	−2.68	−2.87	−1.25
8054722	IL1B	Interleukin 1, beta	−2.94	−2.65	−3.45	−2.45
7963410	KRT6C|KRT6B|KRT6A	Keratin 6C | keratin 6B | keratin 6A	−1.70	−2.58	−2.13	1.16
8144481	DEFB4A	Defensin, beta 4A	−1.94	−2.58	−2.23	−1.11
8149169	DEFB4A	Defensin, beta 4A	−1.95	−2.57	−2.22	−1.12
7968344	ALOX5AP	Arachidonate 5-lipoxygenase-activating protein	−3.18	−2.48	−2.87	−3.08
7920252	S100A7	S100 calcium binding protein A7	−1.76	−2.44	−2.17	1.04
7963421	KRT6A	Keratin 6A	−1.62	−2.40	−2.28	1.25
8028117			−1.92	−2.39	−2.12	−1.99
8066493	SLPI	Secretory leukocyte peptidase inhibitor	−4.08	−2.38	−4.42	−3.72
8095697	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	−2.19	−2.31	−2.59	−2.13
8095680	IL8	Interleukin 8	−2.18	−2.27	−3.41	−3.53
7967318	GPR109A|NIACR1	G protein-coupled receptor 109A | niacin receptor 1	−3.32	−2.25	−3.69	−2.36
7909441	G0S2	G0/G1switch 2	−2.48	−2.24	−2.71	−2.50
7928999	LIPN	Lipase, family member N	−2.19	−2.23	−2.02	−2.04
7920185	LCE3D	Late cornified envelope 3D	−2.24	−2.22	−1.55	−1.00
7922976	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	−2.43	−2.21	−2.09	−1.85
8103226	TMEM154	Transmembrane protein 154	−3.13	−2.15	−1.96	−3.01
8054712	IL1A	Interleukin 1, alpha	−1.85	−2.15	−2.80	−1.49
7914950	CSF3R	Colony stimulating factor 3 receptor (granulocyte)	−3.03	−2.12	−2.19	−2.39
8055980	CYTIP	Cytohesin 1 interacting protein	−2.34	−2.11	−1.88	−2.13

surfaces. Functionally relevant categories inclusive of the extracellular region and matrix, collagen; its fibril organization and response to stress were clearly demonstrated at both implant surfaces for both subjects' group (Table 3).

DISCUSSION

The present study compared the gene expression profiles of implant adherent cells in smokers and nonsmokers during the initial 7 days of peri-implant endosseous healing. Secondarily we investigated the impact of implant surface topography between groups. The choice of time points was taken to detect the very early molecular events and how it maybe potentially affected by smoking. A comprehensive understanding of the complex biological events occurring at this time point in the bone implant interface may ultimately lead to improve biologically driven strategies for enhanced osseointegration. At these time points, processes related to the extracellular matrix organization, angiogenesis, and regulation of the inflammatory processes are activated.[34]

We noted that during the first week of healing, the expression profiles between the 2 groups were very similar suggesting that the systemic impact of smoking is limited at this very early time point. Potentially, detrimental effects are likely to occur at a later stage and upon exposure of the implants to the oral environment. These implants were submerged and never exposed to the oral environment prior to retrieval. The absorption of nicotine through the oral mucosal tissues is pH dependent. Since the pH of tobacco smoke in most cigarettes is acidic, nicotine is primarily ionized[36] resulting in minimal absorption of nicotine from cigarette smoke. Therefore it is also likely that our results reflect a minimal impact from nicotine absorbed directly absorbed through oral tissues.

The systemic effects of smoking on implants are controversial. In a prospective randomized clinical study, Lambert and coworkers reported that the detrimental effects of smoking were evident after the implants were exposed to the oral environment (ie, after second stage surgery).[6] In contrast, chronic exposure to intermittent cigarette smoke or nicotine has been shown to affect peri-implant healing in a rat tibia model as noted by reductions in area of bone deposition within the implant threads,[16] bone density,[17] and bone-to-implant contact.[37]

Our analysis limited to early peri-implant healing indicates that gene expression profiles of implant adherent cells are similar among smokers and nonsmokers. This raises the

TABLE 3

Top 30 Gene Ontology Consortium (GO) terms for differentially regulated genes at Osseospeed (OS) (day 7 vs day 3) in the nonsmoker group with their count in selection/*P*-values in OS, TiOblast (TiO) in both subjects' groups.

GO ID	GO Term	Count in Selection/ *P*-value (Nonsmoking, OS)	Count in Selection/ *P*-value (Smokers, OS)	Count in Selection/ *P*-value (Healthy, TiO)	Count in Selection/ *P*-value (Smokers, TiO)
21158	Extracellular region part	52 (1.08E-20)	58 (1.4318504E-18)	70 (1.1528817E-23)	18 (2.6222415E-09)
3951	Proteinaceous extracellular matrix	27 (3.85E-14)	35 (2.9599606E-18)	30 (1.3128745E-11)	14 (4.652022E-11)
13485	Extracellular matrix	28 (3.85E-14)	35 (3.9120233E-17)	32 (3.778339E-12)	14 (8.0685895E-11)
3949	Extracellular region	62 (3.31E-13)	80 (4.121434E-16)	92 (1.2503183E-17)	19 (2.1903381E-05)
3985	Extracellular space	35 (9.06E-12)	38 (8.378108E-10)	52 (9.404979E-17)	9 (0.011735547)
12780	Extracellular matrix organization	14 (3.45E-09)	16 (9.370151E-10)	12 (5.0762024E-05)	7 (2.957436E-06)
19873	Extracellular structure organization	14 (3.45E-09)	16 (9.370151E-10)	12 (5.0762024E-05)	7 (2.957436E-06)
21157	Extracellular matrix part	14 (1.16E-08)	19 (2.272132E-12)	15 (4.152133E-07)	8 (2.6486893E-07)
12781	Collagen fibril organization	8 (1.75E-07)	8 (1.4044722E-06)	8 (4.1033286E-06)	5 (2.957436E-06)
5061	Response to stress	46 (2.18E-06)	54 (1.5307784E-05)	71 (9.137614E-10)	14 (0.011978871)
3953	Collagen	8 (3.61E-06)	10 (6.480576E-08)	7 (7.323058E-04)	7 (3.833332E-09)
15371	Collagen metabolic process	7 (3.43E-05)	7 (1.4105794E-04)	7 (3.6096672E-04)	—
21000	Multicellular organismal macromolecule metabolic process	7 (5.92E-05)	7 (2.5190072E-04)	7 (5.471943E-04)	—
20977	Multicellular organismal metabolic process	7 (1.64E-04)	7 (5.7112426E-04)	7 (0.0012792945)	—
7176	Tissue development	24 (1.88E-04)	—	27 (0.007247231)	—
9316	Cellular component organization	50 (2.82E-04)	—	—	—
1209	Pattern binding	11 (7.33E-04)	14 (4.3797263E-05)	13 (9.745925E-04)	—
12825	Polysaccharide binding	11 (7.33E-04)	14 (4.3797263E-05)	13 (9.745925E-04)	—
14916	Multicellular organismal process	72 (8.37E-04)	—	105 (5.326255E-04)	—
24906	Anatomical structure development	51 (8.37E-04)	60 (0.0066629495)	76 (5.003017E-05)	—

possibility that the systemic effects of nicotine are noted at a later time point. Indeed, Yamano et al,[38] showed that while no differences were noted on bone-to-implant contact (rat femur model) after 2 weeks of systemic nicotine exposure, significant differences were observed after 4 weeks (late stage) postsurgery. In parallel, they noted significantly decreased expression of *Bmp2, Bsp, Opn, Col2, Cbfa1* in peri-implant tissues in rats exposed to nicotine compared with controls (saline injections) at 4 weeks. This demonstrates that the systemic effects of nicotine on peri-implant healing occur at later stages. Future studies may include greater than 2 weeks' evaluation to investigate other periods of osseointegration (ie, resorptive, quiescent, etc).

Moreover, the effects of smoking may have been negated or delayed by the implant surface topography. In a long-term retrospective study, Balshe et al,[39] compared the survival rates of smooth and rough surface dental implants among smokers and nonsmokers. Smoking was identified as significantly associated with implant failure only in the smooth surface dental implants group. Similar results were reported by Sayardoust et al[40] in patients with periodontitis, where the smokers' likelihood ratio for implant failure was 6.40 for smooth surface implants and 0 for oxidized implants. In addition, Berglundh and coworkers, utilizing TiOblast implants placed in the tibia and femur of rabbits and exposed to either short-term (8 weeks)[19] or long-term exposure (6 months)[41] to nicotine reported that the histometric analysis and the removal torque values were no different between animals exposed to nicotine and controls after 2 and 4 weeks of healing. Moreover, enhanced bone-to-implant contact, biomechanical interlocking, and increased expression of bone-specific gene markers have been shown in implants with moderately rough surfaces and those embellished with nanoscale surface features. Although not investigated in the current study it is possible that any detrimental systemic effects of smoking on peri-implant healing at the molecular level may have been observed if implants with machined surfaces were used.

Also, it is unclear if the age of the population included in both the smoking and nonsmoking groups might have contributed to the results found. In this study, the mean age of smokers was on average 10 years younger than nonsmokers. Further follow-up studies are needed to determine if age influences gene expression profiles of implant adherent cells.

The explanted model with implant adherent cells provides important information critical to driving the process of osseointegration at the implant–bone interface. One limitation to this approach is the scarcity of the RNA quantity on human explanted implants in this population that did not allow further validation of our microarray data using quantitative real time polymerase chain reaction.

Conclusions

Within the limits of the present study, smoking did not seem to alter the early molecular gene expression events occurring at either a microroughened or a nanosurface implant.

Abbreviations

GO: Gene Ontology Consortium
HF: hydrofluoric acid
IRB: Institutional Review Board

OS: Osseospeed
TiO: TiOblast

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