IN VITRO ADHESION OF COMMENSAL AND PATHOGENIC BACTERIA TO COMMERCIAL TITANIUM IMPLANTS WITH DIFFERENT SURFACES

C. PASSARIELLO[1], F. PERA[2] and P. GIGOLA[3]

[1]Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Rome Italy;
[2]Department of Implantology and Prosthodontics, University of Turin, Turin, Italy;
[3]Department of Surgical Specialities, Radiologic and Medico-Forensic Sciences University of Brescia, Brescia Italy

Received July 24, 2012 – Accepted March 8, 2013

Although dental implants have undergone impressive evolution in recent years, periimplantitis still remains a relevant problem and information on the susceptibility of commercial implants to bacterial colonization is insufficient. This work evaluated the susceptibility of different commercial implants to bacterial colonization, to identify key features for good performances. Twenty-four implants, produced with different technologies, were colonized with 9 bacterial strains following pre-conditioning with culture medium, or saliva or serum proteins and adherent bacteria were enumerated by Real Time quantitative PCR. The studied implants differed significantly for susceptibility to bacterial adhesion. Pre-conditioning of surfaces affected adhesion assays in a species specific manner. Although surface topography influenced bacterial adhesiveness, implants produced by different manufacturers with comparable technologies showed great variability of results. These data demonstrate that susceptibility of implants to bacterial colonization is influenced by productive technologies (in a surface topography proportional manner) and by the productive environment. In choosing an implant the clinician should rely upon specific experimental studies, because surface characteristics alone cannot predict susceptibility to colonization by pathogenic bacteria. Tests should include assays performed in the medium of culture and in the presence of serum proteins.

The introduction of dental implants into clinical practice has dramatically modified the approach of dentistry to treatment planning. Osseointegration, as well as creation of a healthy contact area with the mucosal connective tissue, are essential issues for the clinical success of dental implants. Research in the field of implant materials and implant design successfully improved clinical outcomes by allowing fast generation of healthy hard and soft tissues around dental implants (1).

Titanium implants varied from having a smooth machined surface to surfaces treated in order to increase surface topography and enhance intimate contact with bone cells. Different procedures of surface treatments have been studied to favor faster and stronger bone formation (2) allowing more rapid loading of the implant.

Although the number of different methods used to create a rough titanium surface is high, these methods can be summarized into main categories that include: sandblasting, acid etching, electrochemical oxidation and covering with hydroxyapatite or other

Key words: bacterial adhesion, titanium, dental implants, surface topography

Mailing address: Dr. Claudio Passariello,
Dipartimento di Sanità Pubblica
e Malattie Infettive,
Università "Sapienza",
Piazzale Aldo Moro 5, 00185, Rome, Italy
Tel.: +39 06 49914885
e-mail: claudio.passariello@uniroma1.it

0394-6320 (2013)

compounds intended to improve bone integration (3). More recently manufacturers are adopting innovative strategies that create micro and submicro (nano) topographies (4, 5). Commercially available implants frequently adopt combinations of treatments that in many cases are not as well characterized as older ones in respect to surface properties (6).

In the attempt to improve implant surfaces in regard to optimal tissue integration and rapid proliferation of osteoblasts, less consideration has been given to susceptibility of the improved surfaces to bacterial colonization, that constitutes a severe menace for dental implants. In fact, uncontrolled bacterial colonization of implant surfaces coming in contact with the oral cavity leads to the formation of complex bacterial biofilms, and is believed to be the first crucial step of a process that, by triggering inflammatory responses, is able to cause a periimplant mucositis that can further evolve to a periimplantitis (7). Scientific data have clearly related the formation of bacterial biofilms to the onset of periimplantitis (8, 9).

Bacterial biofilms are specialized microbial communities characterized by a peculiar physiology that makes early recognition of biomaterial centred infections a quite difficult issue (10, 11), significantly biasing any option of a simple non-invasive pharmacologic treatment.

Although recent studies demonstrated that titanium surfaces with nanoscale topography decrease bacterial adhesion (5), these effect seems largely susceptible to any minimal alteration. Moreover, most studies that have investigated the susceptibility of different titanium surfaces to bacterial colonization, surfaces with increased topographies and nanometer scale morphologies are more easily colonized by bacteria (12). Unfortunately, these works are mostly performed on samples specifically prepared for research purposes and not on implants coming from production lines. Consequently, though interesting and useful, they cannot allow a comparative evaluation of commercial products that is necessary to the clinician to chose the implant that best fits his needs. This work was consequently aimed to evaluate *in vitro* the susceptibility of commercially available implants with different surfaces to colonization by different bacteria in order to disclose key features that influence this parameter and can be useful for clinicians.

MATERIALS AND METHODS

Dental implants

Twenty-four different commercially available dental implants, with different surface characteristics, were used (Table I). Length, diameter and shape of the implants were selected so that they were the nearest possible to conical 13 x 4.5 mm. Nine implants for each bacterial strain were tested.

Bacterial strains and cultures

Nine reference strains of 8 different bacterial species commonly colonizing implants, possibly causing periimplantitis were used (Table II). All strains were maintained in stock cultures frozen at -80°C in an adequate culture medium (Table II) containing glycerol (20% v/v). For adhesion assays, isolated colonies of each strain were inoculated in the adequate liquid medium and incubated at 37°C with shaking till the mid-logarithmic phase of growth. Bacterial cells were then collected by centrifugation and suspended in fresh sterile medium at $OD_{600nm} = 0.5$.

Adhesion assays

Dental implants were removed from their original package in a sterile class II biohazard cabinet, individually placed in sterile 2 ml polypropylene microcentrifuge tubes, covered with 1.2 ml of sterile culture medium, or sterile heat inactivated foetal bovine serum (PAA Laboratories GmbH, Linz Austria)(FBS), or sterile saliva, and incubated at 37°C for 1 h. Saliva was obtained by paraffin stimulation from five healthy volunteers (having refrained from eating and drinking in the previous 2 hours) and checked for pH being in the range 7.0 to 7.3. Saliva samples were subjected to sonication (1 minute at 30W with refrigeration), filtered through a 70 μm filter (Cell Strainer, Becton Dickinson Italia, Buccinasco, Italy) and centrifuged at 22,000 x g for 60 min at 4°C. Supernatants were pooled, sterilized by sequential filtration through 0.45 μm and 0.2 μm filters, stored at 4°C and used within the next 48 h.

Following contact with the different bacterial suspensions, the implants were transferred to new sterile tubes, washed five times with sterile PBS and further processed for the enumeration of adherent bacteria by quantitative Real Time PCR.

Bacterial DNA extraction

To extract bacterial DNA from lysates of adherent bacteria the Nucleospin Genomic DNA purification Kit (Macherey-Nagel GmbH Düren, Germany) was used. To obtain lysis of bacteria adherent to the surface of implants, 1.2 ml of lysis buffer (20 mM Tris-HCl; 2 mM EDTA;

1% Triton X-100; pH 8.0 supplemented with 20mg/ml lysozyme and 0.2mg/ml lysostaphin) were added to each implant that was then incubated at 37°C for 60 min. Proteinase K was then added and samples were incubated at 56°C until complete lysis was obtained. Following lysis total DNA was purified according to the instructions of the manufacturer. Purified DNA was recovered and stored at -80°C as the template for Real Time PCR reactions. All chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Quantitation of bacterial DNA by Real Time PCR

The quantification of DNA of adherent bacteria in the samples was performed using a Real Time PCR method described previously (13). Quantitative analysis was performed following construction of species specific standard curves using each couple of primers against a serial dilution of the corresponding genomic DNA. A reaction mixture was prepared for each target gene using the Sybr Green Master Mix (Applied Byosystems, Carlbad, CA, USA), with 0.5 μM/L of both primers. Reactions were performed in a volume of 20 μl with 1 μl of each sample as template, using an Applied Byosystems 7300 apparatus. Forty cycles of denaturation at 94°C and annealing/extension at 59°C for 30 seconds were performed, followed by cycles necessary to assess purity of amplification products by construction of the melting curve. Purity of amplification products was assessed following construction of melting curves. Data were reported as number of bacteria detected for each implant.

Statistics

Statistic evaluation of the significance of differences among results of adhesion assays was performed by the Student's *t*-test available in the Microsoft Excel software. Differences yielding values of P in the range >0.01 to ≤ 0.05 were considered significant while differences yielding values of $P \leq 0.01$ were considered very significant.

RESULTS

Characteristics of the tested dental implants

Twenty four commercial dental implants, differing for surface characteristics, were assayed for susceptibility to colonization by nine common bacterial pathogens. Implants differed for surface characteristics. Although most of them (14 of 24, 58.3%) had surfaces resulting from combined physical and chemical treatments, they were divided into 4 groups basing on the nature of the last treatment received (Table I). Consequently, 5 of 24 (20.8%) were classified in the sandblasted group (SB), 8 of 24 (33.3%) in the acid etched group (AE), 3 of 24 (12.5%) in the electrochemically oxidized group (EO) and the remaining 8 of 24 (33.3%) in the group characterized by additional covering (COV) of titanium.

Results of adhesion assays

Results of adhesion assays for all tested strains and implants in the 3 different experimental conditions are reported in Fig. 1 and show that a significant variability exists in susceptibility of the studied implants to bacterial adhesion. Results of adhesion assays were influenced by pre-conditioning of the surface, although not all strains were equally affected (Fig. 1). In fact, FBS significantly enhanced adhesiveness of *S. aureus*, *P. aeruginosa*, *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*, but not of the tested streptococci (Table III). Saliva significantly enhanced adhesiveness of *S. aureus* SA1448 and of all tested streptococci but not of the remaining strains (Table III).

The analysis of cumulative results of adhesion tests after aggregation of implants according to the type of surface treatment showed that implants of the AE, EO and COV groups yielded more uniform results, while great variability was observed in the SB group. Analysis of mean values of adherent bacteria detected in the four groups evidenced that AE and COV implants yielded comparable results while EO showed the best overall results and SB the worst ones (Table IV) although the implant showing the lowest values for 6 of 9 tested bacteria belongs to the SB group (Fig. 1). Considering each group, implant V was the best performing of SB, implant J was the best performing of EO, implant H was the best performing of COV and implant N was the best performing of AE (Fig. 1).

P.aeruginosa showed the highest adhesiveness to dental implants among the tested bacteria, while results of adhesion tests performed with the remaining strains were substantially comparable (Fig. 1 and Table III).

DISCUSSION

Although in a few decades implants have caused a revolution in dental practice, becoming a fundamental tool in the armamentarium of dentists,

Table I. *Principal characteristics of the implants used for bacterial adhesion assays.*

Implant	Dimensions (mm)	Surface treatment	Surface group assigned[a]	Recognition code in figures
Biomet 3i, NanoTite	13x4.0	CaP nanoparticles covering	COV	A
Biomet 3i, Osseotite	13x4.0	CaP Plasma Spray Covering	COV	B
Dentsply Friadent, Ankylos	14x4.5	Sandblasting + high temperature acid etching	AE	C
Prodent Italia, Axial SM	13x4.5	Double acid etching	AE	D
Anthogyr, Axiom	12x4.6	Biphasic CaP Sandblasting + acid etching	AE	E
i-Dent, Biocoin	13x4.5	Double acid etching + CaP nanoparticles covering	COV	F
BioImplant Plus Classic	13x4.0	Sandblasting	SB	G
BioImplant k-Plus HyaloPlus	12x4.0	Hyaluronic acid covering	COV	H
BioImplant k-Plus NgActive	12x4.0	Electrochemical oxidation + CaP covering	COV	I
Astra Tech Osseospeed TX	13x4.5	Sandblasting + Fluoride Electrochemical oxidation	EO	J
BTLock, BT-Tite CV1	13x4.5	Sandblasting + acid etching	AE	K
BTLock, HA coated standard	13x4.5	Plasma Spray + hydroxyapatite covering	COV	L
Leone, Exacone	14x4.1	Sandblasting	SB	M
Megagen, EZ Plus	13x4.1	Hydroxyapatite Sandblasting + acid etching	AE	N
Fornilimplant bifasici classic	12x4.8	Sandblasting	SB	O
Fornilimplant bifasici New	12x4.8	Sandblasting	SB	P
Medical Titanium Biodesign	14x4.1	Al_2O_3 Sandblasting + double acid etching	AE	Q
Intralock System, Intralock Standard	13x4.3	Nanostructured CaP covering	COV	R
Nobel Biocare, Nobelactive	13x4.3	Electrochemical oxidation	EO	S
Sweden & Martina khono DES	13x4.25	1/3 Zirconium Sandblasting + acid etching, 2/3 High roughness Plasma Spray Covering	COV	T
Sweden & Martina Khono ZirTi	13x4.25	Zirconium Sandblasting + acid etching	AE	U
BioSAFin Winsix BioActive Covering	13x4.5	Sandblasting	SB	V
BioSAFin Winsix BioActive Covering SLA	13x4.5	Sandblasting + acid etching	AE	W
BioSAFin Winsix Full Contact Covering	13x4.5	Electrochemical oxidation	EO	X

[a]*Implants were divided into 4 groups basing on the last treatment performed on the surface; SB: sandblasting; AE: acid etching; EO: electrochemical oxidation; COV: covered by different procedures with an extra layer.*

Table II. *Bacterial strains used for experiments.*

Species	Strain	Origin	Medium for growth
Staphylococcus aureus (MSSA[a])	SA1448	Human pharynx	LB[e]
Staphylococcus aureus (MRSA[b])	USA300-0114	Community acquired infection	LB
Streptococcus oralis	DSM 20627	DSMZ[c]	THB[f]
Streptococcus sanguinis	DSM 20567	DSMZ	THB
Streptococcus salivarius	DSM 20560	DSMZ	THB
Pseudomonas aeruginosa	ATCC 27853	ATCC[d]	LB
Aggregatibacter actinomycetemcomitans	DSM 8324	DSMZ	TSBY[g]
Porphyromonas gingivalis	DSM 20709	DSMZ	MWC
Prevotella intermedia	DSM 20706	DSMZ	MWC

[a]Methicillin Susceptible Staphylococcus aureus; [b]Methicillin Resistant Staphylococcus aureus; [c]Leibniz-Institute DSMZ German collection of microorganisms and cell cultures GmbH; [d]American type culture collection; [e]Luria Bertani medium; [f]Todd Hewitt broth; [g]Trypticase Soy broth supplemented with 0.6% yeast extract; [h]Modified Wilkins Chalgren medium (28).

their clinical success is still jeopardized by the onset of periimplant infections occurring at rates ranging 5 to 10% at 5 years (8). Researchers and industries have worked hard to obtain faster osseointegration and early prosthetic charge of implants, by enhancing implant designs and by developing modified titanium surfaces (1). Concerning surfaces, increased topography was demonstrated as the key element to enhance biologic performances *in vitro* (6, 14). Currently used titanium implants display a wide variety of microstructural and chemical characteristics (15). Different subtractive or additive methods, such as machining, blasting, acid etching, electrochemical oxidation, plasma spraying, physical vapor deposition, and adsorption are used to produce titanium implant surfaces with various topographies, oxide thicknesses, crystallinities and compositions, that show different biological behaviors both *in vitro* and *in vivo* (16-18). While each single procedure is subjected to extensive *in vitro* trials, less attention is in general dedicated to its long-term clinical behavior, although available data demonstrate that implant performances are influenced not only by the type of modification but also by the presence of impurities derived from the manufacturing process (1).

Formation of bacterial biofilms at the surface of implants is undisputedly the cause of periimplantitis (8, 9) and remains a relevant clinical problem (7), inducing progressive loss of tissue integration in the almost complete absence of subjective symptoms (10, 11), and offering few options of a simple and non-invasive conservative treatment (19, 20). Nevertheless, implant surfaces were developed paying only minor attention to their susceptibility to bacterial colonization. As a general rule, bacterial adhesiveness is directly proportional to roughness, and smoother surfaces are consequently less prone

Table III. *Adherent bacteria/implant detected by Real Time PCR for each tested strain following pre-conditioning of implants with the culture medium, or FBS or saliva.*

Strain	adherent bacteria/implant Mean (±SD)			P^d		
	MED[a]	FBS[b]	SAL[c]	MED *vs* FBS	MED *vs* SAL	FBS *vs* SAL
SA1448	32334 (±19303)	65229 (±40750)	47744 (±29522)	**$8.4x10^{-4}$**	0.038	0.095
USA300	33114 (±23390)	63870 (±42933)	44299 (±30580)	**0.003**	0.161	0.075
DSM20627	29030 (±18169)	27821 (±16842)	48325 (±37411)	0.812	0.028	0.018
DSM20567	26958 (±16493)	29406 (±19199)	44799 (±33714)	0.638	0.024	0.058
DSM20560	34031 (±20868)	31498 (±18249)	55391 (±38849)	0.657	0.021	**0.009**
ATCC27853	44920 (±22649)	89268 (±487201)	48767 (±24453)	**$2.0x10^{-4}$**	0.575	**$6.9x10^{-4}$**
DSM8324	27681 (±15109)	57085 (±39697)	34681 (±17188)	**0.001**	0.141	0.015
DSM20709	27093 (±16489)	56049 (±38563)	24127 (±15501)	**0.001**	0.524	**$4.8x10^{-4}$**
DSM20706	30587 (±17756)	62432 (±39369)	23836 (±8815)	**$7.5x10^{-4}$**	0.102	**$2.5x10^{-5}$**

Mean values ± Standard Deviation (SD) are reported.
[a]Implants pre-conditioned with the culture medium; [b]Implants pre-conditioned with foetal bovine serum; [c]Implants pre-conditioned with pooled sterilized human saliva; [d]Values obtained by performing the Student's t-test on data from the single implants. Values of P indicating highly significant differences are highlighted in bold, and those indicating significant differences are underlined.

to be colonized (21). Recent research (5) suggests that nanoscale morphologies may decrease bacterial adhesion, although minimal alterations of the structure may result in the opposite effect (12).

Moreover, data available on this subject, including strategies to reduce bacterial adhesion to biomaterials (22, 23) derive from experiments that use specifically-prepared samples, the performances of which are not directly applicable to commercially available products. Clinicians, on the contrary, need information on the behavior and performances of commercial implants. This consideration prompted us to plan experiments to fulfill this need. We consequently analyzed the susceptibility of 24 commercially available implants to colonization by 8 different bacterial species including oral colonizers and species causing periimplantitis.

Implants were selected so that they were very similar in shape and dimensions and included both internationally and locally diffused implants, with representatives of the four main types of surfaces that are actually available for clinical application. Implants with different surfaces, but produced by the same manufacturer, were also included in the study.

Adhesion assays were performed following 3 different pre-conditioning procedures: tests were performed in standard conditions, following treatment of surfaces with culture medium, and in conditions enabling to evaluate the influence of serum and salivary components. Comparison of data obtained in the 3 experimental conditions confirmed that salivary components favored adhesion of streptococci (24) but did not significantly influence adhesiveness of the other tested bacteria, with the exception of *S. aureus* SA1448. Pre-conditioning with serum proteins, on the other hand, significantly enhanced adhesiveness of all tested strains except streptococci. Basing on available data, this effect was expected for *S. aureus*

Table IV. *Mean values of adherent bacteria / implant detected by Real Time PCR for each tested strain following pre-conditioning of implants with the culture medium, and divided according to type of surface processing technique.*

Strain	Adherent bacteria/implant Mean (±SD)						
	SB[a]	AE[b]	EO[c]	COV[d]			
SA1448	48759 (±38669)	28165 (±7690)	22751 (±6286)	29832 (±6352)		SB *vs* AE	**$1.1x10^{-5}$**
USA300	52425 (±47914)	27988 (±8630)	22253 (±5936)	30242 (±6698)		SB *vs* EO	**$5.4x10^{-4}$**
DSM20627	44736 (±37062)	25693 (±6916)	20794 (±6537)	25640 (±2940)		SB *vs* COV	**$7.7x10^{-6}$**
DSM20567	41690 (±33248)	23569 (±7030)	19763 (±4988)	23839 (±2032)	*P*[e]	AE *vs* EO	**$6.3x10^{-3}$**
DSM20560	53435 (±41944)	29807 (±7183)	23601 (±5978)	30039 (±3082)		AE *vs* COV	0.982
ATCC27853	65002 (±44381)	41589 (±10845)	34587 (±6403)	39575 (±7185)		EO *vs* COV	**$5.7x10^{-4}$**
DSM8324	40371 (±31448)	25004 (±4646)	20774 (±2543)	25016 (±3131)			
DSM20709	41866 (±33753)	24427 (±5507)	19698 (±2631)	23300 (±2467)			
DSM20706	47017 (±36296)	27223 (±5294)	23762 (±3706)	26242 (±1976)			
Overall	48367 (±35740)	28163 (±8591)	23109 (±6181)	28888 (±6366)			

[a]*Implants with surfaces finished by sandblasting;* [b]*Implants with surfaces finished by acid etching;* [c] *Implants with surfaces finished by electrochemical oxidation;* [d]*Implants with surfaces finished by covering with different components* [e]*Values obtained by performing the Student's t test on data from the single implants. Values of P indicating highly significant differences are highlighted in bold. Mean values ± Standard Deviation (SD) are reported.*

(25), but not for other tested bacteria including *P. gingivalis* and *A. actinomycetemcomitans* (26, 27). These observations suggest, moreover, that adhesion assays following treatment with saliva can be adequately substituted by the easier to perform standard tests using culture medium, while additional tests carried out following treatment with serum are mandatory to evaluate susceptibility of the surface to colonization by potential pathogens. It must not be forgotten, and it would deserve future evaluation, that different surface treatments could also influence the behavior of different surfaces after moderate-to-long permanence in the aggressive environment of the oral cavity.

As to the main question addressed in this work, significant differences were detected in all experimental conditions among the different tested implants. Analyzing results obtained with single implants, it is evident that some implants are less susceptible to colonization by bacteria (irrespective of the species) and other ones are colonized significantly easier. In an attempt to identify a technology yielding implants less prone to be colonized, we decided to analyze results following aggregation of implants in

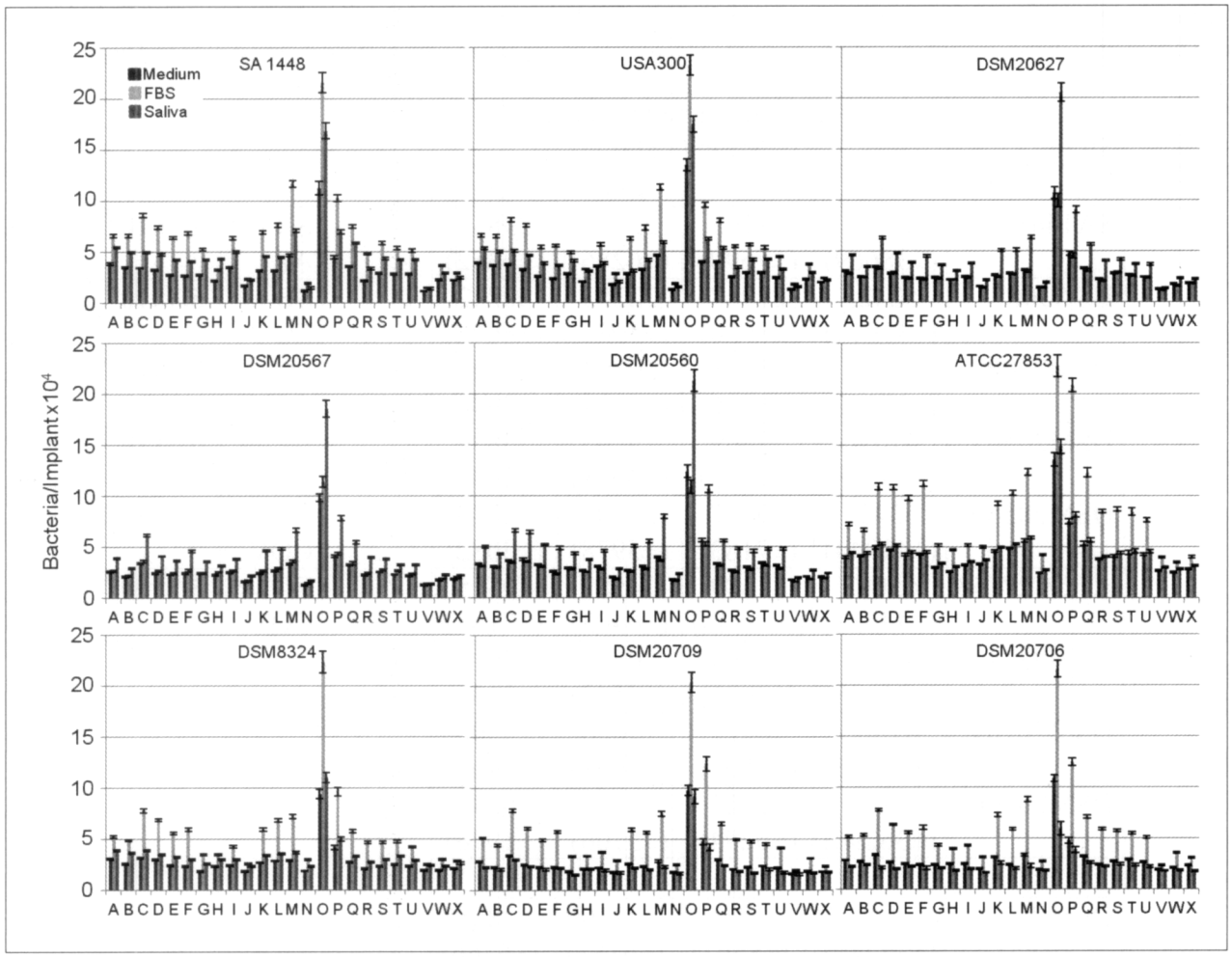

Fig. 1. *Mean values (± Standard deviation) of adherent bacteria detected at the surface of the 24 tested implants, following pre-conditioning of the implant surface with the medium of culture (Medium) (see Table II for details), or foetal bovine serum (FBS), or filter sterilized pooled human saliva (Saliva), with 9 different bacterial strains. Data are means of 3 determinations for each implant and are expressed as bacterial genomes per implants (x10⁴). Correspondence of letters with implants is reported in Table I.*

groups. To this purpose the 24 tested implants were aggregated into 4 groups (namely: SB, AE, EO and COV) basing on the last surface treatment used in their production. Such an aggregation was justified by the consideration that it is arguable that the last treatment performed on a surface should prevail in determining characteristics of this surface in its interaction with biomolecules and cells. Analysis of results following aggregation in groups showed that no single procedure yields one surface that is better than the others although SB surfaces seem to have a less predictable behavior, and include both the best and worst performing implants. Overall, our data suggest that the technique used to enhance surface topography is relevant for susceptibility of the implant to bacterial colonization but that a greater influence is played by the productive environment. In fact, comparison of results obtained with implants characterized by different surfaces but produced by the same manufacturer, hence in a comparable productive environment, have shown that surface topography and bacterial adhesion are directly proportional, while comparative analysis of implants produced by different manufacturers showed that

implants produced by manufacturers with more sophisticated productive facilities are better than others to prevent of bacterial colonization. These data enforce the concept that experimental data on the susceptibility of implants to bacterial adhesion should always be included among biological tests to validate commercial implants.

ACKNOWLEDGEMENTS

This work was supported by a grant from MIUR (Project PRIN 2007LXNYS7_003 granted to CP) and by funds of the University of Brescia granted to PG.

REFERENCES

1. Palmquist A, Omar OM, Esposito M, Lausmaa J, Thomsen P. Titanium oral implants: surface characteristics, interface biology and clinical outcome. JR Soc Interface 2010; 7:515-27.
2. Wennerberg A, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. Int J Oral Maxillofac Implants 2010; 25:63-74.
3. Junker R, Dimakis A, Thoneick M, Jansen JA. Effects of implant surface coatings and composition on bone integration: a systematic review. Clin Oral Implants Res 2009; 20:185-206.
4. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science 2005; 310:1135-38.
5. Singh AV, Vyas V, Patil R, et al. Quantitative characterization of the influence of the nanoscale morphology of nanostructured surfaces on bacterial adhesion and biofilm formation. PLoS ONE 2011; 6(9):e25029.
6. Ellingsen JE, Thomsen P, Lyngstadaas SP. Advances in dental implant materials and tissue regeneration. Periodontol 2000 2006; 41:136-56.
7. Lindhe J, Meyle J. Peri-implant diseases: Consensus report of the Sixth European Workshop on Periodontology. J Clin Periodontol 2008; 35(S):282-85.
8. Mombelli A, Marxer M, Gaberthüel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. J Clin Periodontol 1995; 22:124-30.
9. Pontoriero R, Tonelli MP., Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. Clin Oral Impl Res 1994; 5:254-59.
10. Costerton JW, Montanaro L, Arciola CR. Biofilm in implant infections: its production and regulation. Int J Artif Organs 2005; 28:1062-68.
11. Selan L, Passariello C, Rizzo L, et al. Diagnosis of vascular graft infections with antibodies against staphylococcal slime antigens. Lancet 2002; 359:2166-68.
12. Puckett SD, Taylor E, Raimondo T, Webster TJ. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials 2010; 31:706-13.
13. Passariello C, Puttini M, Virga A, Gigola P. Microbiological and host factors are involved in promoting the periodontal failure of metaloceramic crowns. Clin Oral Invest 2012; 16(3):987-95.
14. Anselme K, Bigerelle M. Topography effects of pure titanium substrates on human osteoblast long-term adhesion. Acta Biomater 2005; 1:211-22.
15. Binon PP. Implants and components: entering the new millennium. Int J Oral Maxillofac Implants 2000; 15:76-94.
16. Lim YJ, Oshida Y, Andres CJ, Barco MT. Surface characterizations of variously treated titanium materials. Int J Oral Maxillof Imp 2001; 16:333-42.
17. Dohan Ehrenfest DM, Coelho PG, Kang BS, Sul YT, Albrektsson T. Classification of osseointegrated implant surfaces: materials, chemistry and topography. Trends Biotechnol 2010; 28:198-206.
18. Mohammadi S, Esposito M, Hall J, Emanuelsson L, Krozer A, Thomsen P. Short-term bone response to titanium implants coated with thin radiofrequent magnetron-sputtered hydroxyapatite in rabbits. Clin. Implant Dent Relat Res 2003; 5:241-53.
19. Selan L, Thaller MC, Berlutti F, Passariello C, Scazzocchio F, Renzini G. Effect of slime production on the antibiotic susceptibility of isolates from prosthetic infections. J Chemother 1989; 1:369-73.
20. Renvert S, Roos-Jansaker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. J Clin Periodontol 2008; 35:305-15.
21. Renvert S, Polyzois I, Claffey N. How do implant

surface characteristics influence peri-implant disease? J Clin Periodontol 2011; 38(S)11:214-22.
22. Bruellhoff K, Fiedler J, Möller M, Groll J, Brenner RE. Surface coating strategies to prevent biofilm formation on implant surfaces. Int J Artif Organs 2010; 33(9):646-53.
23. Hu H, Zhang W, Qiao Y, Jiang X, Liu X, Ding C. Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO(2) coatings on titanium. Acta Biomater 2012; 8(2):904-15.
24. Dorkhan M, Chávez de Paz LE, Skepö M, Svensater G, Davies JR. Effects of saliva or serum coating on adherence of *Streptococcus oralis* strains to titanium. Microbiology 2012; 158(Pt 2):390-97.
25. Wagner C, Aytac S, Hänsch GM. Biofilm growth on implants: bacteria prefer plasma coats. Int J Artif Organs 2011; 34(9):811-17.
26. Guentsch A, Rönnenbeck M, Puklo M, Preshaw PM, Pfister W, Eick S. Influence of serum on interaction of *Porphyromonas gingivalis* ATCC 33277 and *Aggregatibacter actinomycetemcomitans* Y4 with an epithelial cell line. J Periodontal Res 2010; 45:229-38.
27. Kuroda K, Hirano Y, Tamura M, Hayashi K. Inhibitory effect of serum globulins on the adhesion of *Prevotella nigrescens* to hydroxyapatite. J Oral Sci 2003; 45:11-16.
28. Pederson ED, Turner DW, Lamberts BL, Schade SZ. Reducing medium for the cultivation of *Porphyromonas gingivalis*. Microbios 1997; 89(359):119-24.