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Microscopical and microbiologic characterization of customized titanium abutments after different cleaning procedures

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Abstract

Aim: To assess and characterize pollution micro-particles and bacterial growth on customized titanium abutments after steaming, ultrasonic and plasma cleaning treatments.

Materials and methods: Thirty commercially available implant abutments, after customization, were randomly divided into 3 groups of 10 and cleansed by steam (considered as control group), ultrasonic cleaning (test group 1) and plasma of Argon (test group 2). For all specimens, SEM analysis and EDAX microanalysis were performed to count and characterize pollution micro-particles, both on the abutment surface and implant–abutment connection. For the control and test groups, mean values and standard deviations were calculated for number and density of micro-particles. Statistical differences were determined by one-way ANOVA with Scheffe multiple comparison test. The level of statistical significance was set at $P \leq 0.05$. Additional microbiologic analysis was performed to detect bacterial contamination on the abutment surface.

Results: In the control group, the number of micro-particles on average was 117.5, and 14.1, respectively, on the abutment surface and connection. In the test groups, no pollution was revealed on the abutment (average of 1.09 and 1.13 spots, respectively, in test group 1 and test group 2) and connection (1.28 and 1.41, respectively, in test group 1 and test group 2). The analysis of variance (ANOVA) showed a statistically significant difference for all the variables examined. For each variable, at least one of the groups differs from the others. Scheffe multiple comparison test showed that all comparisons for every variables between the control group and both groups are significant, while there were some comparisons between test group 1 and test group 2 that were not significant. EDAX microanalysis identified micro-particles as residual of lubricant mixed with traces of Titanium and other metals. Microbiologic analysis demonstrated the presence of bacterial growth on the abutment surface only in the control group (111.5 ± 11.43 CFU/ml/implant–abutment as mean value). In the test groups, absence of growing microorganisms was found.

Conclusions: This study confirmed that both plasma and ultrasonic treatments can be beneficially adopted for abutment cleaning process after laboratory technical stages, to supposedly favor soft tissue healing and implant–prosthetic connection stability.

For a long time, it has been described that for two step-implants, the relationship between the implant–abutment connection and surrounding hard tissues plays a determinant role in establishing the functional and esthetic prognosis of an implant supported restoration (Abrahamsson et al. 1996). In fact, post-restorative bone resorption was demonstrated to be related, together with mechanical etiology (Isidor 2006), to the bacterial contamination of the implant–abutment junction (Cochran et al. 1997).

However, the stability of the soft tissue implant–abutment interface was demonstrated to affect the preservation of marginal bone as well. In fact, it has been shown how the interactions between cellular components and implant–abutment materials influence the stages of the healing process around implants (Piattelli et al. 2011).

Titanium abutments after customization were demonstrated to present contaminants and debris (2–4 μ in width micro-particles of titanium mixed with lubricant) on the

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surface and at the connection level (Canullo et al. 2012). Presence of such contaminants at the platform–abutment level has been suggested to cause an associated tissue-damaging inflammation, and titanium wear microparticles were demonstrated to activate osteoclastogenesis (Mishra et al. 2011). At the same time, despite tolerances at the implant–abutment connection, micro-particles were demonstrated to affect implant–abutment fit, leading to an increased mechanical stress on connection. This condition may induce preload loss or fracture and cause biological complications due to bacterial penetration within a possible fixture–abutment gap. (Micarelli et al. 2012).

However, a matter of evidence is lacking in the dental implant-related literature in regard to the cleaning protocols after abutment customization and their effects on peri-implant soft and hard tissues. In fact, as osseointegrated implants were used as dental prosthesis' anchorage, the surgical procedure was suggested to be performed with respect to the basic rules of sterility and asepsis (Adell et al. 1985). Controversially, these concepts often were not respected during the second surgical procedure (abutment connection), despite the fact that the titanium abutment was placed at the level of vital tissues.

To prevent this inconvenient, ultrasounds or plasma of Argon cleaning treatments – which are protocols routinely adopted in dental industry to clean fixture and surgical instruments – could be suggested.

Ultrasonic cleaners use high-frequency sound waves to agitate an aqueous or organic medium, which mechanically acts on contaminants adhering to substrates. Ultrasonic treatment does not sterilize surfaces. In fact spores and viruses remain after such cleaning (Jatzwauk et al. 2001).

Plasma cleaning has been loosely defined as a process that uses partially or wholly ionized gas with a roughly equal number of positively and negatively charged particles. It is also known as the "fourth state" of matter, as its properties are similar to those of gases and liquids.

Plasma treatment has been used for several decades to clean unwanted materials away at the molecular level and has been proven to be more efficient than cleaning with wet chemical processes (Vezeau et al. 2000). Two categories of plasma technologies exist: high and low temperature. High-temperature plasma is found at atmospheric pressure, such as torches used in stainless steel deposition. Low-temperature plasmas, used in surface modification and organic cleaning, are ionized gases generated at pressures between 13 and 265 Pa.

Low-temperature plasmas work within vacuum chambers where atmospheric gases have been evacuated below 13 Pa. These low pressures allow for a relatively long free path of accelerated electrons and ions and remove all chemical traces remaining from former treatments. An advantage of the plasma method is the possibility, under appropriate conditions, of achieving such a process at relatively low temperatures ($\leq 50°C$), preserving the integrity of materials (Moisan et al. 2002). In fact, Aronsson et al. (1997) found that plasma of Argon removed all chemical traces left from former treatments and effectively producing cleaner and better controlled surfaces than with other preparation methods.

The present study aims to assess and characterize pollutions and bacterial growth on customized titanium abutments after steaming compared to ultrasonic and plasma cleaning treatments, using SEM, chemical microanalysis and microbiologic analysis

The null hypothesis to verify and confirm was that no differences exist in the pollution density mean values and number of surviving microorganisms among the different experimental groups.

Materials and method

For this *in vitro* study, 30 commercially available titanium grade-five implant abutments, 5 mm in diameter with internal hexagonal implant–abutment connection were used (Premium, Sweden and Martina, Padua, Italy).

Abutments were customized with procedures usually adopted in commercial dental laboratories. Milling and polishing were performed using carbide burs mounted on a miller (BF1, Bredent, Bolzano, Italy): KOMET H 356 RSE (12,000–15,000 rpm) and KOMET H 356RS, (5000 rpm) (Komet Italia, Milan, Italy), respectively. For every abutment, a new bur was adopted. During preparation, an oily lubricant (Bredent, Bolzano, Italy) was used to prevent abutment overheating.

The finishing line was placed from 0 to 1 mm apart from the implant–abutment interface, to simulate every clinical condition.

Then, all samples were notched in the coronal portion for four static points to be set back exactly in the same place once removed.

After preparation, abutments were randomly divided into 3 groups of 10. In the first group, cleaning by steam was performed for 5 s at 4 MPa (VAP 1, Zhermark, Cologne, Germany). Steamed abutments were considered as control group.

The second group underwent ultrasonic cleaning (Sonica S3, Soltec, Milan, Italy) being immersed progressively in three different solutions each time for 10 min at 60°C: an antibacterial detergent (CL4%, Soltec, Milan, Italy), pure acetone and pure ethylic alcohol. After each solution, samples were immersed in demineralized water for 5 min at 60°C (test group 1). The total timing was 45 min.

The remaining ten abutments underwent argon plasma treatment in a plasma reactor (Diener Electronic GmbH, Jettingen, Germany). The treatment conditions were 75 W of power and 10 MPa of pressure for 12 min (test group 2).

Microscopic analysis

All samples were assembled in the surface-analyzing device after steaming (control group) and then after ultrasonic bath (test group 1) and plasma cleaning (test group 2). Sterile forceps were used to avoid contamination.

Samples were subjected to the SEM analysis (Microscope Tescan model VEGA// SBH – Tescan s.r.o, BRNO Czech Republic). Using notches on the coronal portion of the abutment as a reference, four scanning electron micrographs were taken at each step for every abutment, to observe both the prepared and the unprepared zone.

Additional scanning electron micrographs were taken at the platform and the external hexagon, which constitute the coupling zone of the abutment to the implant (implant–abutment connection).

Surface pollution chemical characterization

Surface pollution chemical characterization, according to Sawase et al. (2000), was then investigated by Energy-dispersive X-ray spectroscopy (EDX Genesis 2000 – SiLi Detector CDU, EDAX Inc, Mahwah NJ, USA).

The high-energy electron beam produced by the electronic microscope is known to impinge the sample surface and stimulate the emission of characteristic X-rays from the contaminants. The emitted X-rays detected by EDAX allow to obtain chemical profiles of the different elements on the abutment surfaces. A non-destructive analysis on a microscopic scale was therefore performed. In fact, the atomic concentrations of Ti, O, C and several other elements were examined, and the relative concentrations (in atomic%) of the elements were detected.

The chemical analysis of contaminants was performed with a minimum of 1000 times magnification.

Image analysis

Surface pollution particles of the control and test specimens were then counted and measured in microns on each micrograph. The evaluation of debris was carried out with a 50-times magnification of the whole abutment surface photograph. Pictures were taken at high resolution to allow a correct imaging for enlargement. To evaluate the size, position and the number of spots, enlargement of the SEM images was performed.

The first step was to highlight the pollution spots from the image by coloring them with a different color from the background. Black and white was then vectorialized by creating paths on the spots boundaries. This first step was performed using n image analysis software (Adobe Illustrator, Adobe Inc, San Rafael, CA, USA).

The processed image was then exported into Autocad 2006 (version Z 54.10, Autodesk Inc, San Rafael, CA, USA). Pollution density (calculated as the ratio between polluted surface and total surface) was measured both for the abutment external surface (full image) and the connection.

Additionally, same measurements were assessed for the external portion close to the platform (2 mm in height), considered critical because of the peri-implant tissues proximity (partial image).

Microbiologic analysis

Immediately after microscopical analysis, to evaluate bacterial contamination on implant abutments, under sterile conditions in a laminar flux hood, abutments were transferred to 2-ml micro-tubes, containing 1 ml of trypticase soy broth (TSB). Decimal serial dilutions were made up to 10^{-8}; then, 100 µl of 10^{-7} and 10^{-8} dilutions was plated on trypticase soy agar (TSA) and incubated overnight under aerobic conditions. Colonies grown on agar plates were counted, and the results were expressed as colony-forming units (CFU)/ml/implant abutment. Immediately after, in a second set of experiments, the samples were transferred to 13-ml polystyrene tubes, containing 3 ml of trypticase soy broth (TSB) (BBL, Becton Dickinson and Co., Cockeysville, MD, USA) or alternatively 3 ml of Luria broth (LB). Bacterial growth was monitored following an "over-night" (o/n) incubation both at 30°C and 37°C under conditions of aeration. Bacterial growth was quantified through two approaches, firstly a spectrophotometric reading of the bacterial cultures at 600 nm (SmartSpec, Biorad).

Finally, to characterize the relative amount of Gram-positive and Gram-negative bacteria within the population, Gram-stain protocol was applied according to the manufacturer's instructions (Gram Stain Kit, Sigma Diagnostic, Milan, Italy).

Statistical analysis

Mean values and standard deviations were calculated for each outcome variable (spots and density) and were conducted separately for the control and test (plasma and ultrasonic) groups.

All comparisons, for the three levels of the image (full, partial and connection), for the measures spot and density, were performed byANOVA test with Scheffe multiple

Table 1. **Descriptive statistics (mean and SD) for each group involved in study**

		Full		Partial		Connection	
		Mean	SD	Mean	SD	Mean	SD
Control group	Spot	117.5000	34.8018	58.2000	19.4696	14.1000	6.0268
	Density	0.0054	0.0015	0.0045	0.0009	0.0065	0.0024
Ultrasonic group	Spot	1.0900	0.5152	0.7760	0.3662	1.2810	0.2583
	Density	0.0007	0.0001	0.0001	0.0001	0.0008	0.0001
Plasma group	Spot	1.1348	0.0338	0.9585	0.0754	1.4120	0.2811
	Density	0.0007	0.0000	0.0001	0.0001	0.0009	0.0001

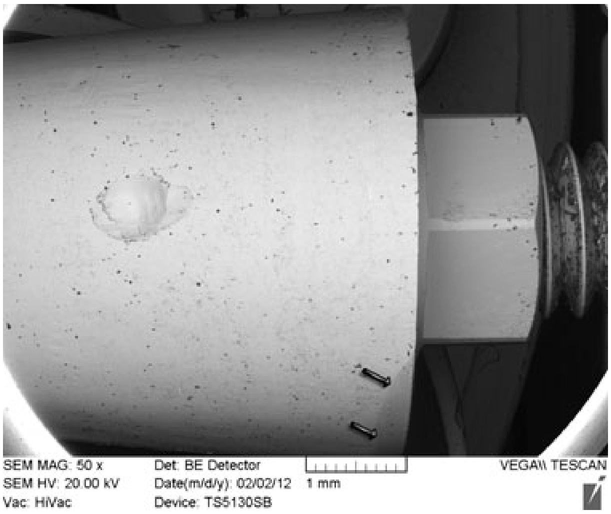

Fig. 1. Control group abutment: several black micro-particles are revealed on the connection and screw (×50). Notch, visible on the coronal portion of the abutment surface, was used to repositioning the sample exactly in the same place. Arrows highlight finishing line.

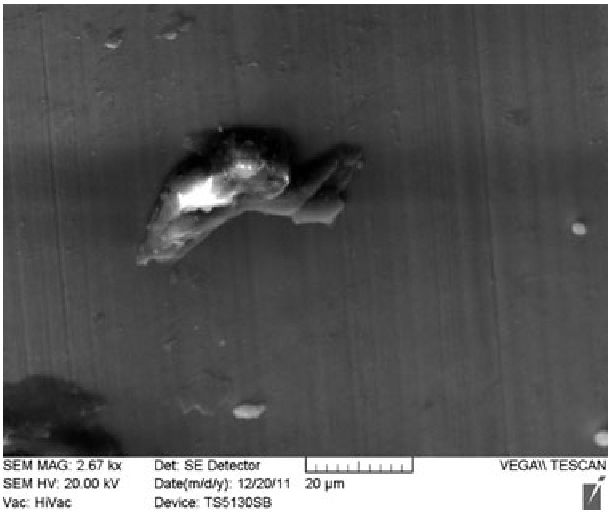

Fig. 2. Higher magnification of Fig. 1 (×1333). Black micro-pollutions were identified as lubricant mixed with titanium wear micro-particles.

comparison. The level of statistical significance was set at $P \leq 0.05$.

Results

Control group

Microscopical analysis and surface pollution chemical characterization

Slightly rough surface was observed on the prepared zone, while on the zone not milled, smooth surface was revealed.

The SEM analysis showed contaminants on the abutment prepared surface and identified several black micro-particles scattered on the abutment surface not interested from preparation. The black micro-particle size ranged between 2 and 3 μ. As resumed in Table 1, spots present on the abutment surface were 117.5 (SD: 34.8) and 58.2 (SD: 19.5) on average, respectively, in the full and partial image. Density ranged between 0.0054 and 0.0045 on average, respectively, in the full and partial image (Fig. 1).

Energy-dispersive X-ray microanalysis confirmed them as the micro-particles of lubricant mixed with titanium, consisting mostly in carbon (60%), oxygen (10%) and titanium (20%).

Additional micro-particles (size ranging between 1 and 2 μ) were found, consisting mostly in carbon (50%) and titanium (40%) (Fig. 4b). Some minor elemental traces of vanadium (2%) and mobildenium (2%) were found (Fig. 2).

Several black micro-particles [mean value of 14.1 spots (SD: 6.0) occupying 0.0115% of the total area] were noted at the connection and screw, identified as lubricant used during abutment preparation (Fig. 3).

Microbiologic analysis

With the aim to evaluate the number of surviving microorganisms, a microbiologic analysis was carried out, and bacterial growth was observed in this group, as clearly demonstrated by the turbidity of the growth medium (Fig. 4a). The average number of surviving bacteria (bacterial cells/ml) was $1.77 \pm 17.4 \times 10^9$, corresponding to an absorbance mean value (OD_{600}) of 3.58 ± 0.42. No relevant differences were observed changing some bacterial growth conditions, such as the temperature of incubation (shifted from 30°C to 37°C) and the medium (LB rather than TSB). These data were confirmed by transferring samples to 2-ml micro-tubes, containing 1 ml of TSB, plating 100 μl of 10^{-7} and 10^{-8} decimal serial dilutions on agar plates and prolonging incubation overnight at 30°C.

A representative image of colonies growth was reported in Fig. 4b, and the average of grown bacterial colonies was 111.5 ± 11.43, expressed as CFU/ml/implant abutment. By applying a Gram-stain protocol, it was demonstrated that Gram-positive bacteria seem to be most abundant, as shown by the violet staining of the colonies, indicative of this class of microorganisms (Fig. 4c). Also, analyzing the morphology of the colonies, it could be speculated that the main

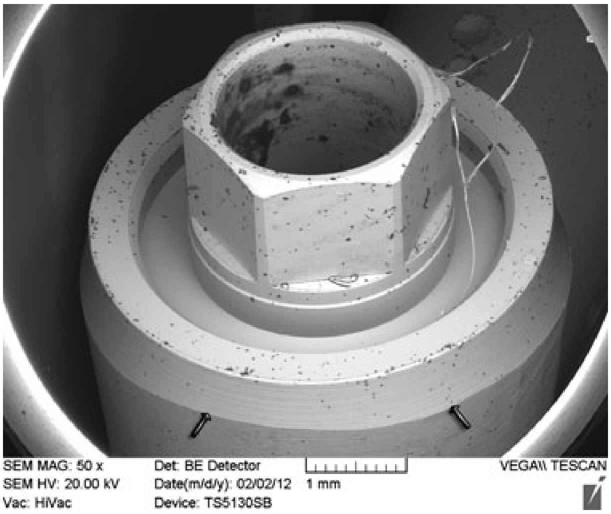

Fig. 3. Control group connection: several contaminant micro-particles are clearly visible, identified as lubricant used during abutment preparation (×50). Arrows highlight finishing line.

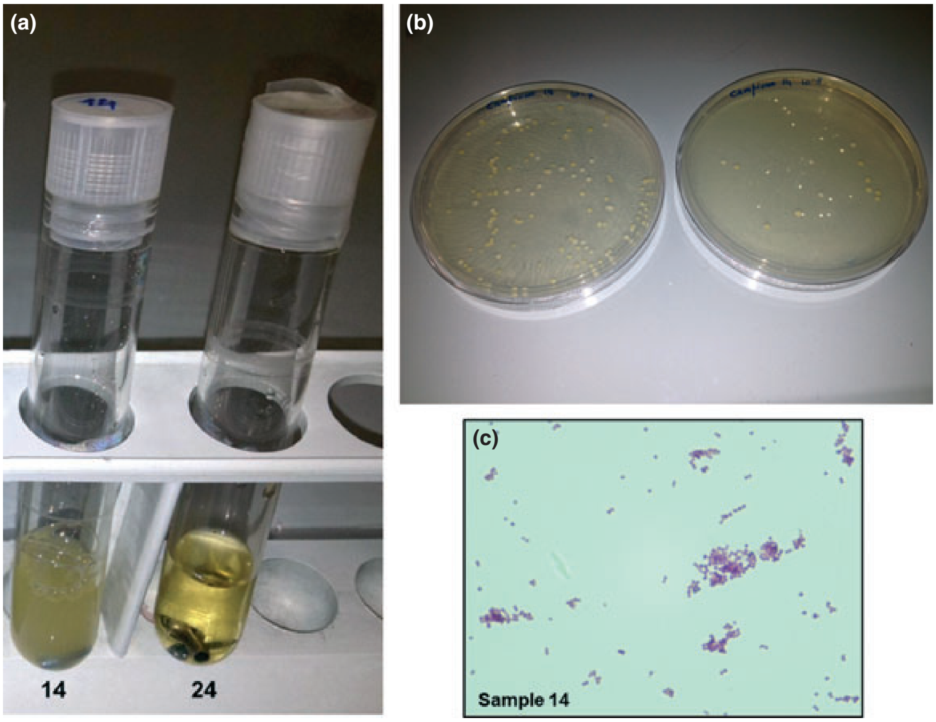

Fig. 4. Analysis of bacterial colonization and growth on implant abutments. Representative images of bacterial growth on abutments, following overnight incubation at 30°C in trypticase soy broth (a) and agar plates (b), respectively. In the figures, we reported one contaminated sample (14) and another one (24) in which no bacterial growth was observed, as negative control. (c) A representative image of Gram staining on bacterial cultures, grown at 30°C, under aeration conditions. The red/violet staining of the colonies is indicative of Gram-positive bacteria. Magnification: ×100.

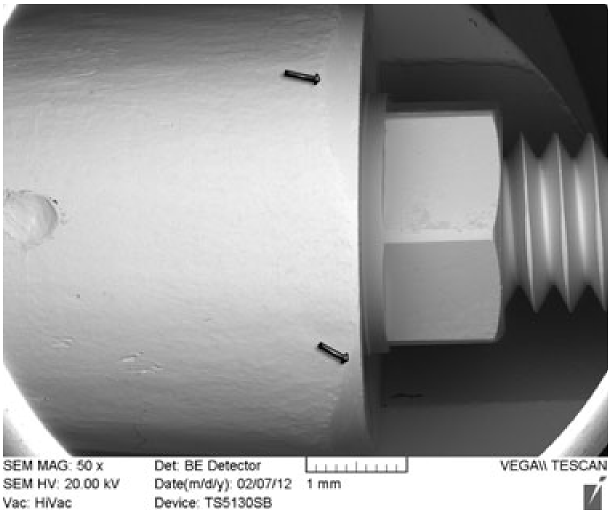

Fig. 5. Test group 1 abutment (ultrasonic cleaning): no pollution is revealed on the abutment surface, connection and screw (×50). Arrows highlight finishing line.

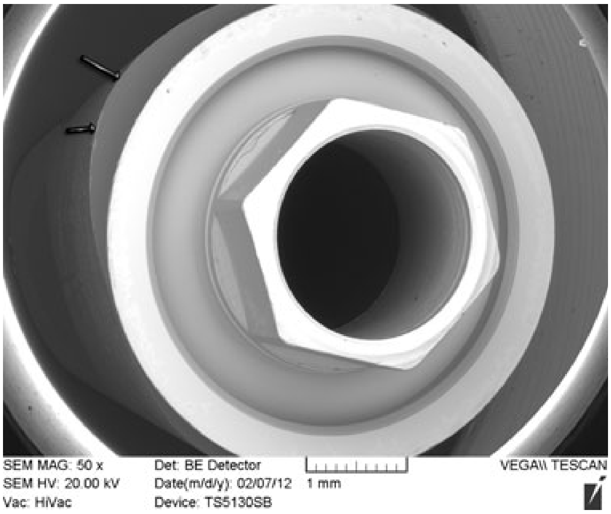

Fig. 6. Test group 1 connection (ultrasonic cleaning): no pollution is revealed (×50). Arrows highlight finishing line.

represented bacterial genera belong to *Staphilococcus* and *Streptococcus*.

Test group 1 (ultrasonic cleaning)

Microscopical analysis and surface pollution chemical characterization

As resumed in Table 1, no pollution was revealed on the whole abutment surface at the SEM analysis [mean value of 1.09 (SD: 0.51) and 0.78 (SD:0.37) spots, respectively, in the full and partial image, with a density ranging between 0.0007 and 0.0001, respectively, in the full and partial image] (Fig. 5).

Similar conditions were detected at the internal connection and abutment screw [mean value of 1.28 spots (SD: 0.25) covering 0.0008% of the surface] (Fig. 6).

Microbiologic analysis

In this group, bacterial growth was not observed, as shown by the absence of turbidity of the growth medium (Fig. 4c).

Test group 2 (plasma cleaning)

Microscopical analysis and surface pollution chemical characterization

As resumed in Table 1, at the SEM analysis, no pollution was revealed on the abutment surface [mean value of 1.135 (SD: 0.33) and 0.958 (SD:0.07) spots, respectively, in the full and partial image, with a density ranging between 0.0007 and 0.0001, respectively, in the full and partial image] (Fig. 7).

Similar conditions were detected at the internal connection and abutment screw [mean value of 1.41 spots (SD: 0.28) covering 0.0009% of the surface] (Fig. 8).

Microbiologic analysis

Also in this group, bacterial growth was not observed, as shown by the absence of turbidity of the growth medium (Fig. 4c).

Statistical analysis

As resumed in Table 2, the analysis of variance (ANOVA) for the two parameters (spot and density) between the control group and the two test groups (plasma and ultrasonic) shows significant differences at all levels of the image (full, partial and connection).

Moreover, Scheffe multiple comparison (Table 3) highlights the differences between control group and both test groups and shows that no statistical differences are detected between test group 1 and test group 2.

Discussion

The aim of the present study was to analyze the pollutants and bacteria on titanium grade-5 abutments after customization and before the clinical application. In fact, the most part of the prosthetic components available in the market is mainly composed of Ti6Al4V (grade-5 titanium alloy) because of its greater yield strength and fatigue properties than pure titanium, but same cell reactions.

The results of this *in vitro* study, according to Canullo et al. (2012), confirmed the presence of pollutions on the abutment surface, connection and screw following traditional milling procedure, even after cleaning by steam.

The presence of contaminants at the platform–abutment level has been addressed as a possible cause for different direct and indirect biologic and biomechanical responses:

- As direct response, it must be noted that at the early stages of peri-implant tissue healing process, contaminants might negatively influence the interactions between cellular components and materials, as these interactions are mediated by the state of the surface (Piattelli et al. 2011).
- Additionally, Mishra et al. (2011) longitudinally described that metallic micro-pollutions could be associated to a tissue-damaging inflammation with consequent osteoclastogenesis.
- As indirect response, it can be supposed that microbiological factors (enhanced plaque accumulation due to

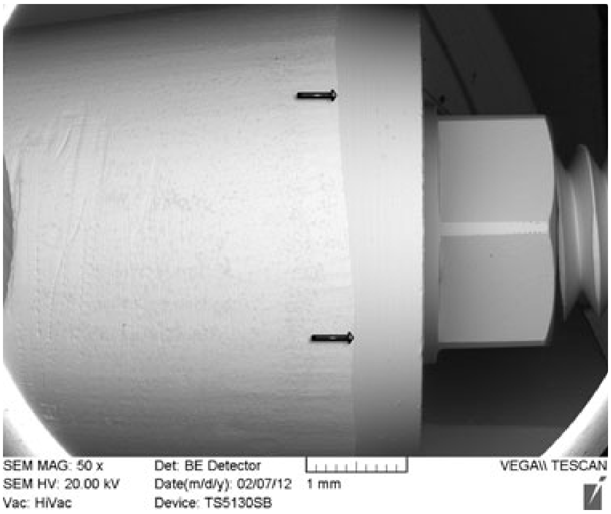

Fig. 7. Test group 2 abutment (plasma cleaning): no pollution is revealed on the abutment surface, connection and screw (×50). Arrows highlight finishing line.

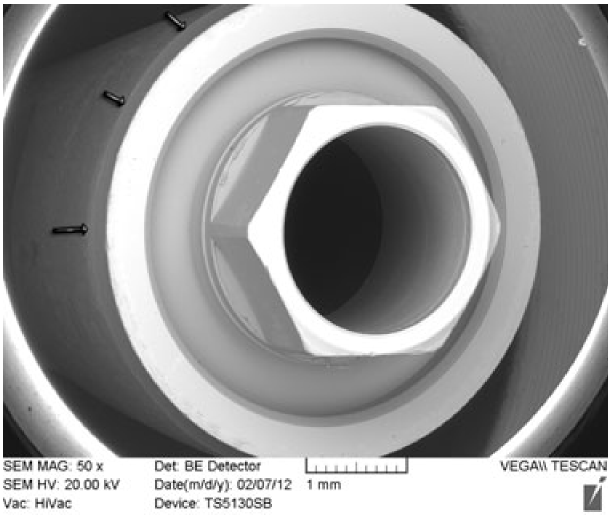

Fig. 8. Test group 2 connection (plasma cleaning): no pollution is revealed (×50). Arrows highlight finishing line.

micro-pollutions) may longitudinally affect the stability of peri-implant tissues. In fact, contamination is recognized to affect the titanium oxide layer (Ehrenfest et al. 2010), and according to Teughels et al. (2006), it enhanced roughness of the abutment surface. Moreover, its chemical composition has a significant impact on the amount and quality of plaque formation.

- Furthermore, analyzing the contamination from a mechanical point of view, such debris and oxide layers may affect mechanical stability of the implant–abutment connection, increasing implant–abutment micro-gap (Van Assche et al. 2011). In fact, despite the tolerances between implant and abutment, presence of contaminant on the connection and screw could negatively affect preloading and thus mechanical stability (Carr et al. 1993).

Although the longitudinal impact of pollutions has not already been clinically demonstrated [except by a pre-clinical short-term study (Canullo & GÖtz 2012)], cleaning protocols could supposedly enhance the implant prosthesis integration. In the present study, two of them (ultrasonic bath and plasma cleaning) were adopted and compared with traditionally used steaming (control group); a largely significant difference was found in regard to contaminants and presence of bacterial contamination.

For the test group 1, the almost complete cleaning obtained could be explained by the sequence of the ultrasonic baths. The nature of the substrate and contamination are key factors in selecting the chemistry cleaning for ultrasounds (Bentley & Sarll 1995). Temperature also plays a crucial role in the cleaning process. In fact, the number of cavitation bubbles increases proportionally to the temperature. This happens up to about 60°C, beyond which cavitation begins to decline and stops completely when the liquid's boiling point is reached (Reichert & Schultz 1998). In fact, as EDAX analysis demonstrated that pollutions on the abutment surface and connection are micro-particles of lubricant mixed with titanium and minimal metallic traces, in the present study, samples of test group 1 were subject to ultrasonic bath in an antibacterial detergent, pure acetone and pure ethylic alcohol, every step for 10 min at 60°C.

The results showed almost complete removal of contaminant both at the surface, connection and screw together with absence of bacterial growing.

Abutments of test group 2 were subject to plasma of argon treatment in a plasma reactor. This treatment provokes a chemical reaction between the gas used and surface contaminants, resulting in their volatilization and removal from the plasma chamber (Youngblood & Ong 2003). However, although the method has been fairly widely used in biomedical research, there is a lack of information available about how plasma process parameters can be optimized for surface cleaning and preparation of biomaterials for clinical use (Carlsson et al. 1989). In fact, care must be taken in the selection of such cleaning process parameters to ensure that pollutions are completely removed (Michaels et al. 1991; Swart et al. 1992). In the present study, the treatment conditions were 75 W of power and 1 MPa of pressure for 12 min, and according to Aronsson et al. (1997), the results showed that all contaminant microparticles and microbiologic component were removed, leading to cleaner and more well-controlled surfaces.

At the end of the study, no statistically significant difference was found between ultrasonic and plasma cleaning, highlighting the efficacy of both treatments. In fact, the both tested cleaning treatments allow for restoring the abutment surface characteristics to pre-customization conditions, as reported by Sawase et al. (2000).

However, it should be underlined that the abutments used in the present study had a smooth surface topography and a conical macro-geometry. These characteristics could be a favoring factor for cleaning methods

Table 2. **The analysis of variance (ANOVA) for the two parameters (spot and density) between the control group and the two test groups (plasma and ultrasonic)**

	ANOVA				
	Sum of squares	df	Mean Square	*F*	Sig.
Spot full					
Between groups	90307.166	2	45153.583	111.819	0.000
Within groups	10902.899	27	403.811		
Total	101210.1	29			
Density full					
Between groups	0.000	2	0.000	93.850	0.000
Within groups	0.000	27	0.000		
Total	0.000	29			
Spot partial					
Between groups	21913.814	2	10956.907	86.683	0.000
Within groups	3412.858	27	126.402		
Total	25326.672	29			
Density partial					
Between groups	0.000	2	0.000	232.538	0.000
Within groups	0.000	27	0.000		
Total	0.000	29			
Spot connection					
Between groups	1084.431	2	542.215	44.605	0.000
Within groups	328.212	27	12.156		
Total	1412.643	29			
Density connection					
Between groups	0.000	2	0.000	57.356	0.000
Within groups	0.000	27	0.000		
Total	0.000	29			

efficacy, thus explaining the absence of statistically significant differences.

From a microbiologic point of view, data of this *in vitro* study demonstrated presence of bacterial contamination only in the control group, confirming the potential risk to insert a contaminated abutment after re-opening in contact with vital tissues. At the same time, absence of microbiologic growth after cleaning protocols in the test groups was shown. However, under the present study experimental conditions of temperature and aeration, several bacteria species are unable to grow, such as anaerobic or "uncultivable" bacteria. This experimental limitation allows to affirm that the heterogeneity of bacterial populations responsible for abutments contamination is much larger than the one observed.

This could explain the absence of difference between test groups, although the Literature demonstrated that ultrasonic cleaning does not allow sterilization (Muscarella 1998), while plasma treatments activate

Table 3. **Scheffe multiple comparison to test the differences between control group and both test groups**

	Scheffe multiple comparison						
						95% Confidence interval	
Dependent variable	(I) Group 1	(J) Group 1	Mean difference (i–j)	Std. error	Sig.	Lower bound	Upper bound
Spot full	Control	Ultrasonic	116.410	8.987	0.000	93.134	139.686
		Plasma	116.365	8.987	0.000	93.089	139.641
	Ultrasonic	Control	−116.410	8.987	0.000	−139.686	−93.134
		Plasma	−0.045	8.987	1.000	−23.321	23.231
	Plasma	Control	−116.365	8.987	0.000	−139.641	−93.089
		Ultrasonic	0.045	8.987	1.000	−23.231	23.321
Density full	Control	Ultrasonic	0.005	0.000	0.000	0.004	0.006
		Plasma	0.005	0.000	0.000	0.004	0.006
	Ultrasonic	Control	−0.005	0.000	0.000	−0.006	−0.004
		Plasma	0.000	0.000	1.000	−0.001	0.001
	Plasma	Control	−0.005	0.000	0.000	−0.006	−0.004
		Ultrasonic	0.000	0.000	1.000	−0.001	0.001
Spot partial	Control	Ultrasonic	57.424	5.028	0.000	44.401	70.447
		Plasma	57.242	5.028	0.000	44.219	70.264
	Ultrasonic	Control	−57.424	5.028	0.000	−70.447	−44.401
		Plasma	−0.182	5.028	0.999	−13.205	12.840
	Plasma	Control	−57.242	5.028	0.000	−70.264	−44.219
		Ultrasonic	182	5.028	0.999	−12.840	13.205
Density partial	Control	Ultrasonic	0.004	0.000	0.000	0.004	0.005
		Plasma	0.004	0.000	0.000	0.004	0.005
	Ultrasonic	Control	−0.004	0.000	0.000	−0.005	−0.004
		Plasma	0.000	0.000	1.000	−0.001	0.001
	Plasma	Control	−0.004	0.000	0.000	−0.005	−0.004
		Ultrasonic	0.000	0.000	1.000	−0.001	0.001
Spot connection	Control	Ultrasonic	12.819	1.559	0.000	8.781	16.857
		Plasma	12.688	1.559	0.000	8.650	16.726
	Ultrasonic	Control	−12.819	1.559	0.000	−16.857	−8.781
		Plasma	−0.131	1.559	0.996	−4.169	3.907
	Plasma	Control	−12.688	1.559	0.000	−16.726	−8.650
		Ultrasonic	131	1.559	0.996	−3.907	4.169
Density connection	Control	Ultrasonic	0.006	0.001	0.000	0.004	0.007
		Plasma	0.00563000	0.00060784	0.000	0.00405567	0.00720433
	Ultrasonic	Control	−0.00564600	0.00060784	0.000	−0.00722033	−0.00407167
		Plasma	−0.00001600	0.00060784	1.000	−0.00159033	0.00155833
	Plasma	Control	−0.00563000	0.00060784	0.000	−0.00720433	−0.00405567
		Ultrasonic	0.00001600	0.00060784	1.000	−0.00155833	0.00159033

ultraviolet photons and radicals, eliminating spores and bacteria (Moisan et al. 2002).

However, for a more global evaluation on the clinical effect of tested cleaning protocols, it should be speculated that soft tissue response to titanium abutment surface is not only influenced by its cleanliness: tissue–material interface is, in fact, related to the type of bonding between macro- and micro-molecules of the biologic site and the outer layer of the material; this bond is influenced both by macroscopic characteristics (roughness, pollution) and the microscopic composition of the outermost layer (material biochemistry and wettability) (Kasemo & Lausmaa 1988). Cleaning with plasma of argon is supposed also to have effect on the state of the titanium. In fact, plasma treatment is thought to activate the surfaces at the atomic and molecular level, producing hydrophilic surfaces and enhancing their wettability (Swart et al. 1992)

Accordingly, several authors have confirmed that plasma treatment of the titanium implant surface can affect osteoblast adhesion, activating the surface and thus enhancing the osteoblast adsorption on titanium (Hauser et al. 2009; Junker et al. 2009; Tavares et al. 2009).

Theoretically, positive effects of glow discharge techniques could be applied also to the abutment: mostly at the early stage of wound healing, faster molecular and cellular adhesion on plasma-cleaned titanium abutment in the supracrestal level might lead to a stronger fixation of the connective tissue collar and possibly preventing epithelial downgrowth.

Surface activation and sterilization, together with the cleaning effect, could enhance peri-implant soft and hard tissues stability, allowing wound healing that establishes an effective interface between living tissues and a foreign body. From a clinical point of view, although only in a pre-clinical short-term study with a limited sample size, this approach (abutment cleaning using plasma of Argon) was demonstrated to be effective in peri-implant bone preservation (Canullo & GÖtz 2012).

However, it must be taken into account that the afore-mentioned cleaning processes should be performed immediately before the abutment connection. In fact, the high surface energy following cleaning procedures could provoke a recontamination of the abutment, as concluded by Vezeau et al. (2000).

Despite the positive outcomes of this *in vitro* study and the preliminary clinical feedback, future studies should aim to confirm the clinical relevance of plasma of argon cleaning and whether titanium–tissue interface following such procedures may histologically lead to better tissue healing, to prevent peri-implant disease at short, medium and long term.

Additionally, to increase the knowledge on the effectiveness of cleaning protocols, other materials, such as zirconia or titanium grade 4, should be tested for plasma of argon and ultrasounds.

Conclusions

The results of present study confirmed a great difference in the surface contamination (wear micro-particles and bacteria) between the control group (treated by vapor steaming) and the test groups (cleaned by ultrasounds and plasma of argon). It could be concluded that both plasma and ultrasounds can be positively adopted for abutment cleaning process after technical stages. In fact, statistically significant difference between the test groups was not found. It should be underlined that the abutments used in the present study had a smooth surface topography and conical macro-configuration; these characteristics could be a favoring factor for cleaning methods efficacy, explaining the lack of difference between the two test groups.

It could be supposed that the extreme contaminants and pollution reduction following the tested treatments might clinically both optimize peri-implant tissue healing and minimize post-healing bacterial colonization and inflammation. At the same time, it could be also hypothesized that contaminant and pollution reduction could improve implant–abutment connection matching and stability. However, both hypotheses must be validated through clinical trials.

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