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Decontamination of titanium implants using physical methods

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Abstract

Objectives: Current decontamination methods of titanium (Ti) implant present limited success in achieving predictable re-osseointegration. We hypothesized that even though these techniques could be useful in elimination of bacteria, they might be unsuccessful in removing organic contaminants and restoring the original surface composition. The aim of this study was to assess the effect of four decontamination methods on the surface chemistry and bacterial load of biofilm-contaminated implant surfaces in order to improve implant surface decontamination.

Material and methods: The ability of clinically available methods such as metal and plastic curettes, Ti brushes and Er: YAG laser to decontaminate Ti implant surfaces was assessed. Surface morphology, chemical composition and properties of machined Ti discs (\varnothing 5.0 and 1.0 mm thick) were analysed before and after oral biofilm contamination using scanning electron microscope and X-ray photoelectron spectroscopy. The presence and viability of bacteria were evaluated with live–dead assays.

Results: Biofilm contamination created an organic layer rich in hydrocarbons and bacteria that covered entirely the Ti surfaces. This organic layer has tightly adhered to Ti surfaces and could not be completely removed with any of the methods assessed. Ti brushes achieved greater elimination of organic contaminants and bacteria than curettes and Er: YAG laser; however, none of them was able to restore the original surface chemistry. Alternatively, Er: YAG laser-treated surfaces showed the lowest live-to-dead bacterial ratio.

Conclusions: Ti brushes were more effective than curettes (metal or plastic) and Er: YAG laser in decontaminating Ti implant surfaces, although none of these techniques was able to completely eliminate surface contamination. Er: YAG laser was more effective than curettes and Ti brushes in killing the biofilm bacteria.

Peri-implantitis is an inflammation of tissues surrounding dental implants associated with bleeding, suppuration and bone loss, which eventually results in implant failure (Lindhe & Meyle 2008; Zitzmann & Berglundh 2008). It affects up to 47% of implant patients (Derks & Tomasi 2015) and up to 43% of the implants placed (Figuro et al. 2014). Peri-implantitis is associated with accumulation of microbial biofilm on the exposed implants surfaces, mainly Gram-negative anaerobic microbiota (Hultin et al. 2002; Mombelli & Décaillot 2011).

Many techniques have been assessed and used to manage peri-implantitis. They include non-surgical and surgical decontamination of implants with mechanical instruments, antimicrobial therapies or lasers (Renvert et al. 2008; Subramani & Wismeijer 2012). Bone regenerative and supportive

therapies have also been used in combination with surgical decontamination to enhance the bone re-osseointegration (Claffey et al. 2008; Renvert et al. 2009).

Mechanical decontamination involves scaling and polishing of contaminated implants surfaces using curettes, polishing brushes, ultrasonic devices or air-abrasive powder systems (Schwarz et al. 2005; Gosau et al. 2010). These methods demonstrate success in debriding the contaminated surfaces, but they also cause damage of the implants microstructures. To avoid surface damage, modified tips and gentle abrasive powders were introduced to clean implants. Nevertheless, the success of the mechanical methods has been always limited by their inability to access deep and narrow bony defects (Augthun et al. 1998). Lasers have also been used to control peri-implantitis especially

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carbon dioxide (CO₂) and erbium-doped yttrium–aluminium–garnet (Er: YAG) lasers, with better decontamination results obtained by the latter (Matsuyama et al. 2003; Yamamoto & Tanabe 2013). The cost of laser therapies, however, should be weighed because their use has not shown additional benefits over the cheaper traditional mechanical treatments (Renvert et al. 2008).

Currently, a gold standard for the management of peri-implantitis does not exist. It seems that so far the proposed treatments cannot achieve complete debridement of the bony defect or decontamination of the implant surfaces (Schwarz et al. 2012). Indeed, re-osseointegration fails to occur on implant surfaces exposed to bacterial contamination following traditional treatment of peri-implantitis lesions. However, re-osseointegration can be consistently achieved in sites previously affected by peri-implantitis as long as a pristine implant surface is used (Persson et al. 2001). This indicates that in peri-implantitis, the quality of the Ti surface determines whether re-osseointegration will occur or not. Moreover, these findings could suggest that restoring the implant surfaces to their original condition could be of great importance for achieving true re-osseointegration.

Also, the analytical techniques used in most decontamination studies are not sensitive enough to evaluate the removal of the implant surface contaminants. The majority of these studies assessed bacterial removal at a macroscopic level using light and fluorescence microscopy, or at a microscopic level using scanning electron microscopy (SEM; Kim et al. 2011; Schmage et al. 2011; Idlibi et al. 2013; John et al. 2014). Others used colony-forming unit and bacterial smear tests to examine the presence of viable bacteria and their regrowth after treatment (Kreisler et al. 2002; da Silva et al. 2005). However, even though these qualitative examinations provide valuable information on the presence and viability of bacteria, they offer no clue on the ability of the decontamination techniques to remove bacterial toxins or residual biofilm. The assessment of bacterial organic products on titanium (Ti) implant surfaces might require a higher magnification at the nanoscale level.

More importantly, despite the importance of surface chemistry in implants osseointegration (Sul et al. 2004), no emphasis has been given to the chemical changes that might occur to implants surfaces after decontamination. Therefore, it is critical to investigate in depth the effect of the

physical decontamination methods on surface contaminants, and their ability to create an environment suitable for re-osseointegration.

X-ray photoelectron spectroscopy (XPS) was used in this study to identify and quantify the elements present on Ti surfaces (outermost 5–10 nm) and their chemical state. XPS has been widely used for the chemical analyses of Ti surfaces (Kang et al. 2009; Sahrman et al. 2013); however, it has barely been used to assess decontamination of Ti implants. XPS is a very sensitive technique that can measure surface elements including the adsorbed hydrocarbons. Hence, it could detect any change in the surface elemental composition due to contamination, and give an extremely accurate evaluation on the degree of surface cleanliness after decontamination. We hypothesized that analysing the surface chemistry of Ti surfaces before and after decontamination with physical methods could accurately assess their ability to remove surface contaminants. Therefore, we aimed at evaluating the effect of four commonly used decontamination methods on the surface chemistry and bacterial load of biofilm-contaminated Ti implants.

Material and methods

The study protocol was approved by the Ethics Board of McGill University (application 14-464 GEN). Four healthy non-smoker subjects volunteered for this study and signed an informed written consent before participation. A total of 48-machined Ti discs (Institut Straumann AG, Basel, Switzerland; 5.0 mm in diameter and 1.0 mm in thickness) were used in the experiments directly after removal from the original packaging without further processing. All discs were characterized at 3 time points, as received from the company, after contamination, and after decontamination (Fig. 1).

Samples contamination

Dental biofilm was developed on the machined discs using intraoral maxillary splints following a previously described protocol (Gosau et al. 2010; Idlibi et al. 2013). Maxillary impressions were taken to fabricate acrylic splints to which Ti discs were fixed; each splint accommodated 12 discs. Participants were asked to wear the splints/discs for 24 h and only remove them for eating or drinking while keeping them in phosphate buffered saline. The splints/discs were then collected and stored for further analysis.

Decontamination procedures

The contaminated discs were randomly and equally allocated to four treatment groups (Fig. 1). The treatment groups were cleaned and instrumented as follows:

- **Metal Curettes:** Stainless steel metal curettes (Gracey 5/6; Hu-Friedy, Chicago, IL, USA) were used to scale the samples from bottom to top under water irrigation (Duarte et al. 2009).
- **Plastic Curettes:** Manual plastic curettes made of high-grade resin (Implacare™ II; Hu-Friedy) were also used in a scaling mode with water irrigation (Duarte et al. 2009).
- **Titanium brush:** Rotary brushes made of titanium (Ti brush, 2.5 mm in diameter and 8 mm in length; Institute Straumann AG, Basel, Switzerland) were used at a rotation speed of 920 rpm under irrigation with water (John et al. 2014).
- **Laser:** Erbium: yttrium–aluminium–garnet laser device (AdvErL Evo Er: YAG; J. Morita, Irvine, CA, USA) emitting pulsed infrared radiation at a wavelength of 2940 nm was used following the manufacturer's recommendation. Laser parameters were set at 100 mJ/pulse and a pulse rate of 20 pulses/s. The samples surfaces were irradiated using C600F fiberoptic tip (J. Morita) at an incidence angle of 45° and a distance of 0.5–1 mm from the sample surface. To cover the whole sample surface, the tip was moved from the bottom to the top of the disc in parallel motion at a constant speed. Water was irrigated at a rate of 5 ml/min.

The cleaning with curettes and brushes was performed homogeneously over the entire surfaces with a constant force of 0.23 ± 0.05 N that was calibrated and controlled using a mechanical testing system (MACH-1; Biomomentum Inc., Laval, QC, Canada) before each cleaning procedure. The time needed to complete the removal of biofilm upon visual examination was measured. One skilled operator (A.A) carried out all the cleaning procedures in the same session to assure the reproducibility of the treatments.

Surface analysis

As XPS analysis would be affected by live–dead bacterial assays on the surface, contaminated Ti discs of each splint were randomly and equally allocated to two main groups; therefore, a total of 24 discs were allocated to each group. One group was used to evaluate the change in surface chemistry using

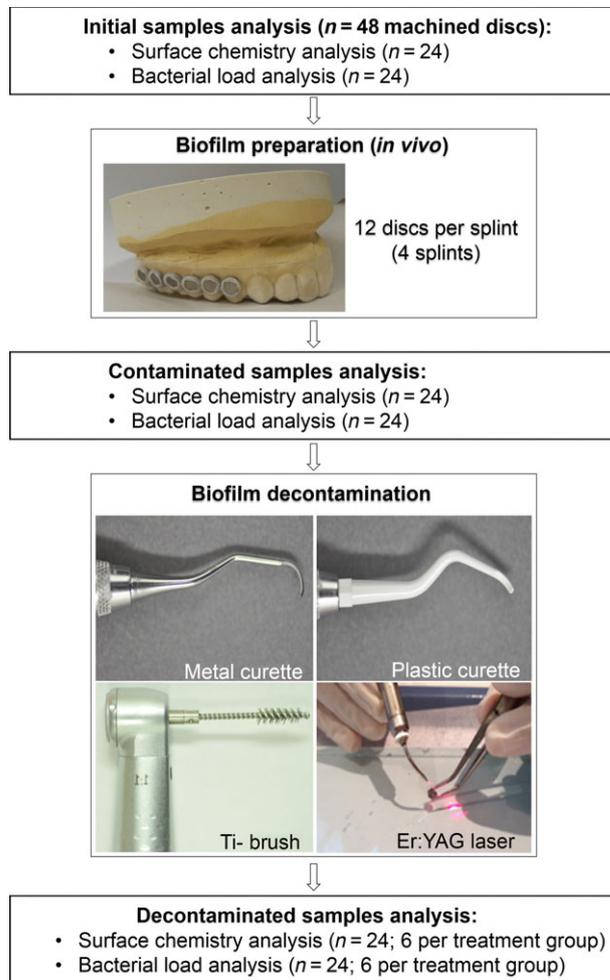


Fig. 1. Flow chart of material and methods.

XPS, and the other group was used to assess the change in bacterial load using live-dead assays and SEM (Fig. 1). The discs were evaluated as received from the company, after contamination and after decontamination using the following techniques:

- X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectrometer (Thermo Fischer Scientific Inc., East Grinstead, UK) was used to analyse the surface chemistry of all samples ($n = 6$ for each treatment group) as previously described (Alageel et al. 2015). The instrument is equipped with a monochromatic Al $K\alpha$ X-ray source (1486.6 eV [λ], 0.834 nm) and an ultrahigh vacuum chamber (10^{-9} torr). The size of the analysed spot was 400 μm . Survey scans were recorded on three different spots on each Ti disc, over the range of 0–1200 eV with a pass energy of 200 eV and a resolution of 1.0 eV. Elements quantification, binding energies and peak areas were acquired using the Avantage analysis

software (5.932v; Thermo Fisher Scientific, Waltham, MA, USA).

- Live/dead bacterial assays and Fluorescence Microscopy (FM)

LIVE/DEAD BacLight bacterial viability kit (L7012; Molecular Probes Inc., Carlsbad, CA, USA) and fluorescence microscopy were used for the quantitative assays of bacteria on both contaminated and decontaminated discs ($n = 6$ for each treatment group). The live/dead stain was prepared by diluting 1 μl of staining component A (SYTO 9; excitation [λ] = 485 nm, emission = 498 nm) and 1 μl of staining component B (propidium iodide; excitation = 535 nm, emission = 617 nm) in 1 ml of distilled water. Discs were placed in 48-well plates and covered with 500 μl of the reagent mixture before incubating them at room temperature and in the dark for 15 min. Each disc was then carefully positioned on a glass slide, covered with component C (mounting oil) and stored in the dark at 4°C until further processing.

Samples were evaluated under a fluorescence microscope (Carl Zeiss Microscopy GmbH, Gottingen, Germany) operated with a ZEN image processing software (Carl Zeiss Microscopy GmbH), using an AxioCam digital camera (MRm Rev. 3; Carl Zeiss Microscopy). For each disc, fluorescent images of five randomly selected sites were captured with a 20 \times objective. Live (fluorescence green) and dead (fluorescence red) bacterial cells in the same microscopic field were viewed separately with different fluorescence filters and then digitally combined into one picture. Means of green, red and total fluorescence per the microscopic field area ($448 \times 335 \mu\text{m} = 0.15 \text{mm}^2$) were then calculated using Cell Profiler image analysis software (Broad Institute of MIT and Harvard, MA, USA).

- Scanning Electron Microscope (SEM)

Surface morphology of clean samples was scanned using a field emission SEM (FE-SEM S-4700, Hitachi, Japan) without further preparation ($n = 2$). The contaminated and decontaminated samples ($n = 5$) were fixed in 2.5% glutaraldehyde (PAA Laboratories GmbH, Pasching, Austria) for 2 h then dehydrated using ascending series of ethanol concentrations (30–100 v/v %) for 15 min each. After that, samples were dried using critical point drying (Ladd Research Critical Point Dryer, Williston, VT, USA) and mounted on SEM-sample stubs where they were sputter-coated with gold and examined. SE mode with and acceleration voltage of 20 kV were selected for analysis, and $\times 10,000$ magnification was chosen for the direct comparison of all samples.

Statistical analysis

The study was designed based on the results of previous studies that demonstrated a significant difference in the cleaning effectiveness of different decontamination techniques using a group of six Ti discs per technique (Schmage et al. 2011; Park et al. 2013). Accordingly, a total of 24 Ti discs were used ($n = 6$ per group) to compare changes in the surface chemistry induced by the four tested decontamination techniques. Same number of discs was used to evaluate changes in the bacterial load (secondary outcome) induced by the same decontamination techniques.

For the statistical analysis, SPSS software (version 22; SPSS Inc., IBM Corporation, Armonk, NY, USA) and Origin (version 9.0; Origin Laboratory, Northampton, MA, USA) were used; describing the Ti discs as statistical units. Means, medians and standard deviations were calculated for all data. The

percentage changes in the chemical composition and bacterial load due to decontamination were also calculated for all groups (Tables S1–S4). Significant differences between groups were assessed using a mixed model with one within factor (time) and one between factor (technique) followed by a post hoc testing with Bonferroni's correction for multiple comparisons. Results were considered statistically significant at a $P < 0.05$.

Results

The time needed for cleaning the samples visually with the metal and plastic curettes was 90.0 ± 4.0 and 90.2 ± 3.1 s, respectively, while it was 60.5 ± 3.5 s for Ti brushes and 49.7 ± 1.6 s for Er: YAG laser.

Surface chemistry

Surface chemistry of Ti samples before and after decontamination was analysed using XPS (Fig. 2). The surfaces of as-received samples were examined directly after removing the sterile package, and it consisted primarily of four elements: oxygen ($43.6 \pm 1.6\%$),

carbon ($38.7 \pm 2.4\%$), Ti ($16.0 \pm 0.8\%$) and nitrogen ($1.7 \pm 0.5\%$). Biofilm contamination significantly increased the carbon and nitrogen levels to $73.8 \pm 1.6\%$ ($P < 0.001$) and $7.8 \pm 0.8\%$ ($P < 0.001$), respectively, at the expense of a decrease in the oxygen ($18.1 \pm 1.1\%$; $P < 0.001$) and Ti levels ($0.2 \pm 0.08\%$; $P < 0.001$), indicating that the organic compounds completely covered the underlying Ti surfaces.

On the other hand, all decontamination methods were able to significantly increase the levels of oxygen to $23.5 \pm 4.7\%$ with metal curette ($P < 0.01$), $27.5 \pm 3.9\%$ with plastic curette ($P < 0.001$) and $39.4 \pm 3.5\%$ with Ti brush ($P < 0.001$) except the laser, the change was not significant ($20.4 \pm 2.5\%$; $P > 0.05$). All methods induced significant decrease of carbon levels in comparison with their levels on biofilm-contaminated surface (metal curette: $65 \pm 6.8\%$, $P < 0.01$; plastic curette: $58.1 \pm 6.2\%$, $P < 0.001$; Ti brush: $43.1 \pm 5.9\%$, $P < 0.001$; laser: $66.6 \pm 3.6\%$, $P < 0.05$). With regard to Ti element, only plastic curette and Ti brush techniques significantly increased its levels compared to

that on contaminated surfaces ($4.4 \pm 2.1\%$, $P < 0.001$ and $9.7 \pm 2.6\%$, $P < 0.001$, respectively). However, the change induced by metal curettes and laser treatments was not significant ($2.3 \pm 2.2\%$ and $0.5 \pm 0.3\%$; $P > 0.05$, respectively). Ti brush was the only method that decreased the levels of nitrogen and this change approached the acceptable significance level ($5.9 \pm 2.0\%$; $P = 0.054$). Obviously, no method was able to restore surface elements to their levels prior to contamination.

Fig. 3 shows the comparisons of the Ti surfaces chemistry between the different decontamination techniques (curettes, Ti brush and laser). Ti and oxygen levels were significantly higher ($P < 0.01$), while carbon and nitrogen levels were significantly lower ($P < 0.01$) in Ti brush-treated samples than that in all other groups. This could indicate that the Ti brushes removed the surface contaminants and exposed the underlying Ti surface. Laser treatments showed the lowest surfaces changes in favour of decontamination ($P < 0.01$).

Bacterial assays

The surfaces treated with metal curettes and Ti brushes showed a comparable number of attached bacteria ($74.9 \times 10^3 \pm 21.6 \times 10^3/\text{mm}^2$ and $70.9 \times 10^3 \pm 31.7 \times 10^3/\text{mm}^2$, respectively), which was not significantly different from that found on uncontaminated surfaces ($24.3 \times 10^3 \pm 19.5 \times 10^3/\text{mm}^2$; $P > 0.05$). Alternatively, the surfaces treated with lasers did not show any change in the number of total bacteria ($268.7 \times 10^3 \pm 45.3 \times 10^3/\text{mm}^2$; $P > 0.05$) with respect to that on the contaminated samples ($296.8 \times 10^3 \pm 16.1 \times 10^4/\text{mm}^2$), but they demonstrated a significant increase in the number of dead bacteria ($403.2 \times 10^3 \pm 26.8 \times 10^3/\text{mm}^2$; $P < 0.001$), thus a decrease of live/dead ratio to 0.3 ± 0.1 (Figs 4 and 5). Fluorescence images (Fig. 6a) showed that the surfaces treated with Ti brushes were almost as clean as uncontaminated surfaces while those treated with laser were entirely covered with dead bacteria (fluorescence red).

Surface morphology

Scanning electron microscopy images of the contaminated surfaces showed a homogenous biofilm layer that completely masked the machining marks of the Ti discs (Fig. 6b). After decontamination, surfaces treated with Ti brushes demonstrated obvious morphological changes while surfaces treated with laser showed residues of bacteria and degraded biofilm. These observations support the XPS

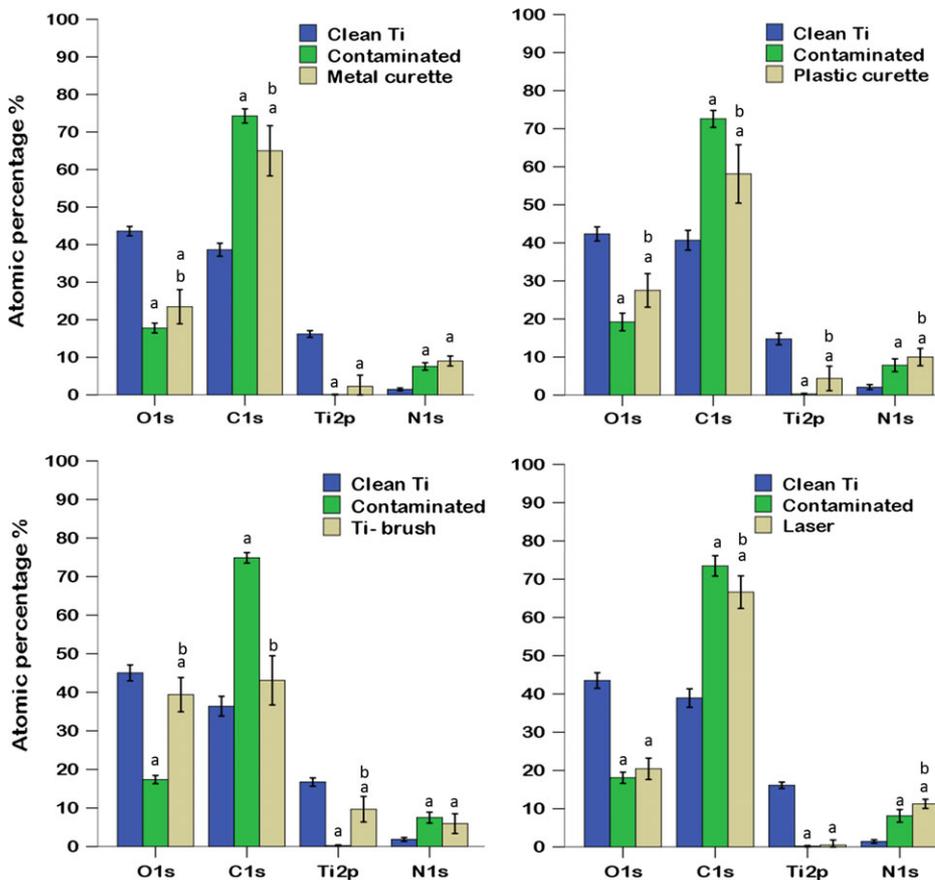


Fig. 2. Bar charts illustrating the elemental composition of titanium surfaces before and after decontamination as detected with X-ray photoelectron spectroscopy (XPS) low-resolution scans. a: Significantly different from clean Ti and b: significantly different from biofilm-contaminated group ($P < 0.05$).

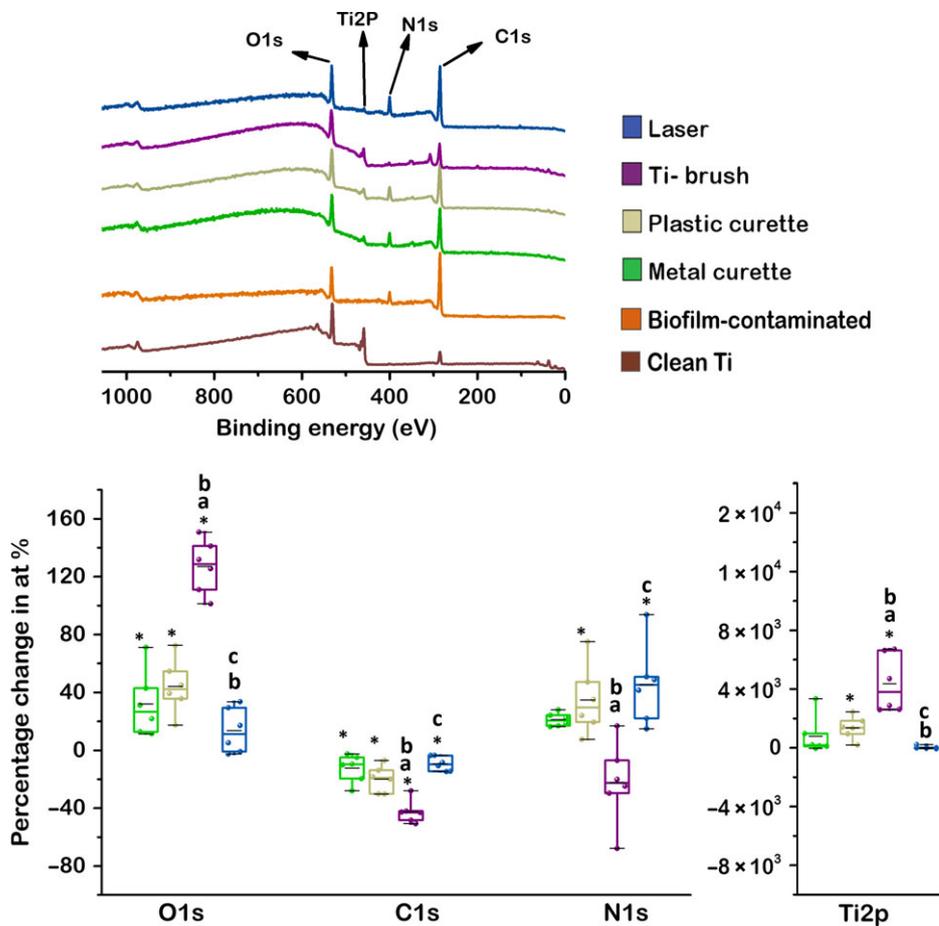


Fig. 3. X-ray photoelectron spectroscopy surveys (top) and box plots overlapped with scatter plots (bottom) illustrating the comparison of the percentage change in the elemental composition of titanium surfaces between different decontamination methods. *Significantly different from biofilm-contaminated group; a: significantly different from metal curette group; b: significantly different from plastic curette group; and c: significantly different from Ti brush group ($P < 0.05$).

data and bacterial assays, confirming that the laser was not able to remove bacteria and other surface contaminants. Photographs of the surfaces treated with metal or plastic curettes showed pronounced scratch lines while those taken for Ti brushes and laser-treated samples showed clean surfaces free of scratches (Fig. 6c).

Discussion

This study provides a comparison assessment on the effect of four commonly used decontamination techniques on the surface chemistry, morphology and bacterial load of biofilm-contaminated Ti. Our results demonstrated the superiority of Ti brushes in mechanical decontamination and Er: YAG laser in killing bacteria.

In this study, we used *in vivo* biofilm model because it offers the opportunity to evaluate implant surfaces in realistic clinical

conditions; formation of composite plaque, co-adherence of microorganisms and salivary pellicle under the removal forces of salivary flow and chewing activities (Rimondini et al. 2002). Several *in vitro* biofilm models have been tested and validated to study the implant surface bacterial interactions (Bürgers et al. 2010; Al-Radha et al. 2012; Schmidlin et al. 2013). This includes for instance the commonly used microtiter plate-based systems (Coenye & Nelis 2010). However, the complex structure of biofilm, the dynamics of its pathogenicity and ecological determinants are not precisely simulated with these models (Blanc et al. 2014; Sánchez et al. 2014).

Most studies assessed the decontamination outcomes based on the evaluation of Ti surface morphology or bacterial removal (Idlibi et al. 2013; John et al. 2014). However, there has been little emphasis on evaluating the removal of other surface contaminants at the molecular and atomic levels. SEM is useful

to visualize the surfaces morphology and the presence of biofilm but this technique does not quantitatively or accurately measure the complete biofilm removal or the changes in the implant surfaces after treatment (Lu et al. 2012). Moreover, contamination of the implant surfaces reduces the surface free energy and hinders implant biocompatibility (Louropoulou et al. 2014), which could negatively affect re-osseointegration. Consequently, this study focused on the quantitative assessment of the Ti surfaces chemistry using XPS and on the evaluation of changes that might occur due to decontamination. This would provide a clear understanding on how different decontamination techniques interact with the biofilm accumulated on Ti surfaces, and their ability to restore the original surface properties.

The surface of the clean samples presented high levels of carbon, although the samples were directly examined after unpacking. This result is in agreement with previous studies reporting that surface carbon levels are higher on machined surfaces than on rough surfaces (Morra et al. 2002). It could be attributed to contamination during the process of machining and polishing, in which the surfaces were in contact with the machining tools and organic lubricating solutions. The carbon levels could further increase due to the unavoidable adsorption of airborne hydrocarbons on the Ti surfaces (Morra et al. 2002; Cassinelli et al. 2003).

Exposure of Ti surfaces to the oral plaque for 24 h was sufficient to allow the formation of a homogenous biofilm that completely covered the surfaces of all samples as shown on SEM and fluorescence images (Fig. 6). This observation corroborated the findings of previous studies that used the same method and time for Ti contamination (Scarano et al. 2004; Schwarz et al. 2005). XPS data further supported these findings and showed a significant increase in the concentration of organic compounds (expressed as higher carbon and nitrogen), resulting in lower Ti and oxygen concentrations. Similar results have also been reported for failed implants contaminated with microbial biofilm (Shibli et al. 2005).

Four decontamination methods that are clinically implemented in the treatment of peri-implantitis were evaluated. The methods included conventional mechanical instruments (metal and plastic curettes), a new mechanical instrument (Ti brush) and Er: YAG laser. Despite the complete removal of surface bacteria by both metal curettes and Ti brushes (Fig. 4), the XPS data showed that

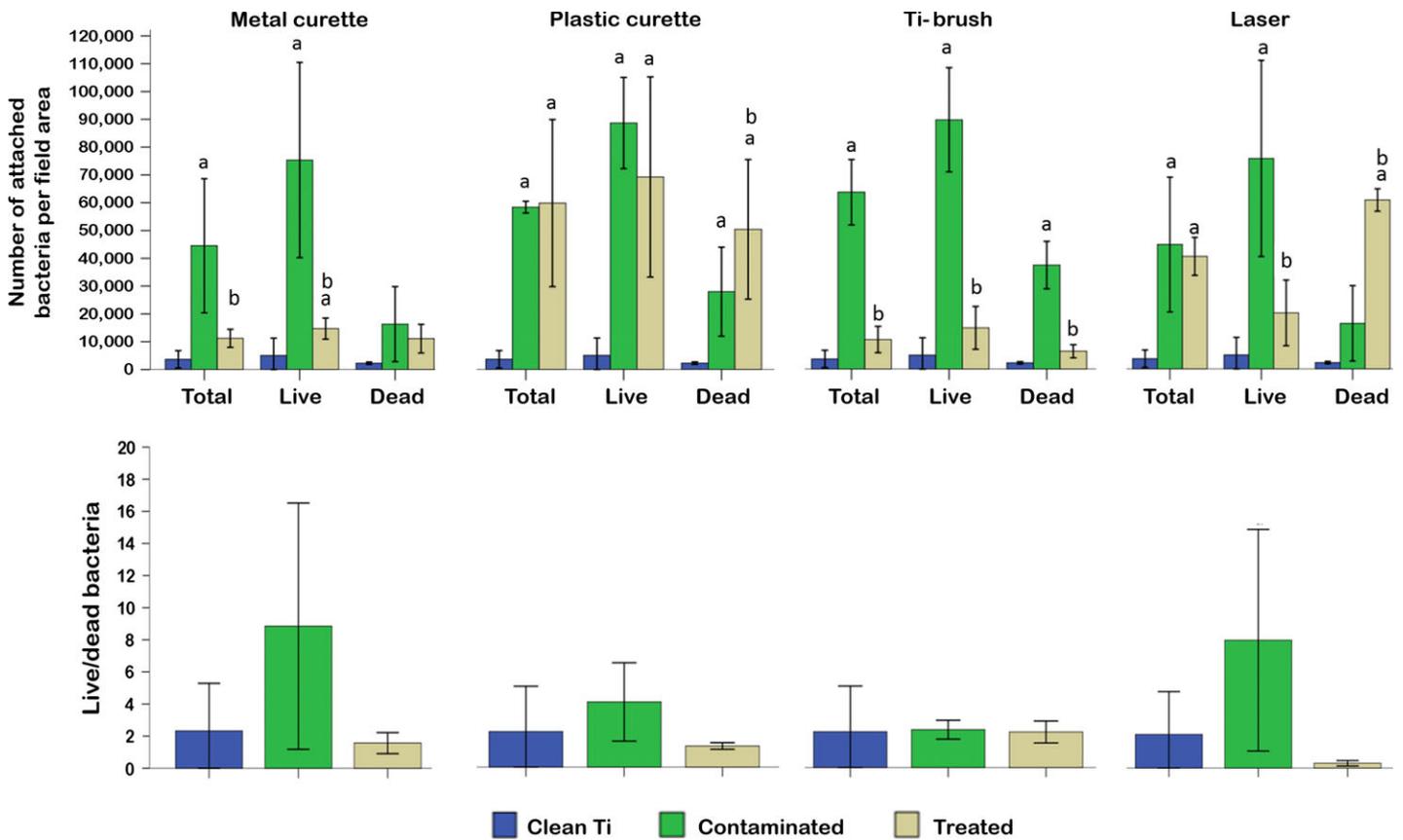


Fig. 4. Bar charts illustrating the number of attached bacteria (per field area of 0.15 mm²) and their viability on titanium surfaces before and after each decontamination method. a: Significantly different from clean Ti and b: significantly different from biofilm-contaminated group (*P* < 0.05).

none of these methods was able to completely decontaminate the samples and restore the surface chemistry to its original condition prior to biofilm contamination. These results emphasized the importance of using XPS analysis to evaluate the decontamination outcomes owed to its unique ability to detect surfaces contaminants. Moreover, our results seem to indicate that the failure of the above-mentioned decontamination techniques to achieve complete re-osseointegration (Schwarz et al. 2006a,b; Takasaki et al. 2007) could be attributed to their inability to completely remove the organic residuals from the surfaces.

Both types of curettes (metal and plastic) were very limited in their ability to remove organic contaminants, and also they induced visible surface scratches. Metal curettes were able to remove more bacteria than plastic curettes, but they induced more surface scratches. These results are in agreement with previous reports that indicated the effectiveness of metal curettes in removing soft biofilm from SLA surfaces, while the plastic curettes were not effective in removing bacteria from polished or rough implant surfaces (Louropoulou et al. 2014). In addition, surface scratches, surface damage and increased

surface roughness have all been commonly reported on machined surfaces after instrumentation with metal curettes (Louropoulou et al. 2012).

Ti brushes were introduced to decontaminate peri-implant lesions, and it has been

claimed that they are able to easily access and disinfect narrow peri-implant defects due to their capability to adapt closely to the implant microstructure (Duddeck et al. 2012; Wohlfahrt & Lyngstadaas 2012). However, prior to our study, the cleaning effectiveness of Ti

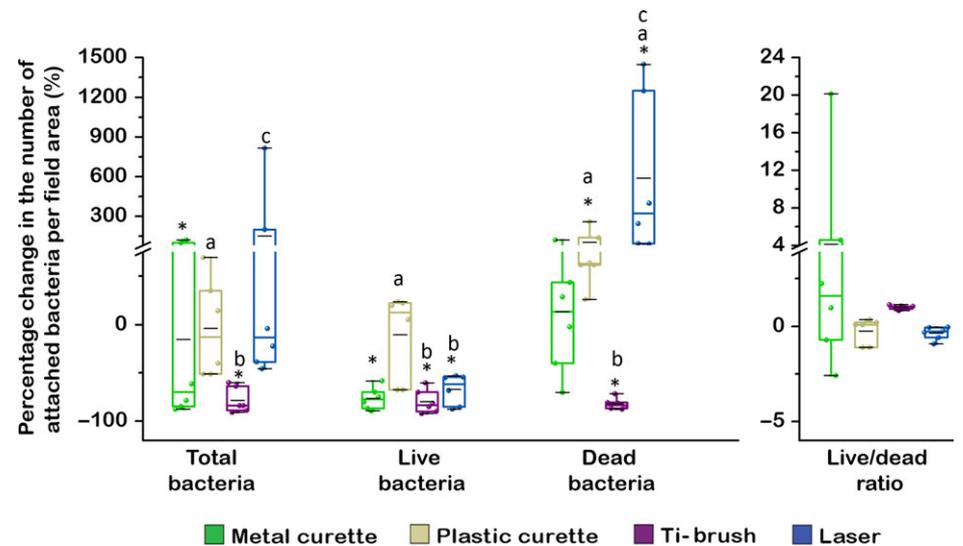


Fig. 5. Box plots overlapped with scatter plots comparing the percentage change in the number of live and dead bacteria (per field area of 0.15 mm²) between different decontamination methods. *Significantly different from biofilm-contaminated group; a: significantly different from metal curette group; b: significantly different from plastic curette group; and c: significantly different from Ti brush group (*P* < 0.05).

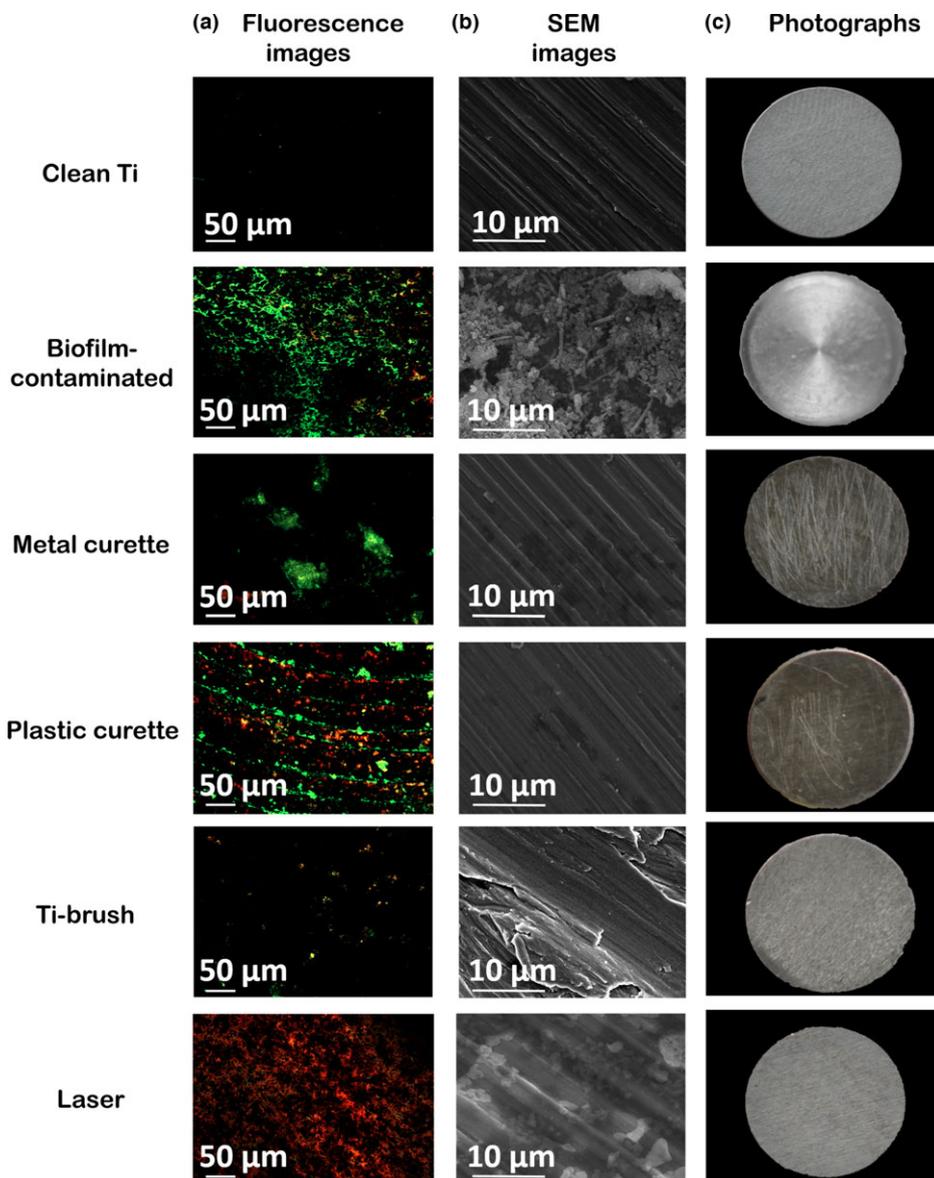


Fig. 6. (a) Fluorescence (live/dead staining) images of bacteria on titanium surfaces, (b) scanning electron microscope images illustrating the morphology of the titanium surfaces and (c) photographs of titanium surfaces.

brushes has not been demonstrated and the literature is scarce on the decontamination of implant surfaces using Ti brushes. To our knowledge, only previous study that has tested the decontamination outcomes of Ti brushes in comparison to metal curettes, demonstrated Ti brushes were superior to metal curettes in their ability to remove plaque while preserving Ti surfaces (John et al. 2014). In this previous study, the residual plaque area on the Ti surfaces was calculated using histomorphometric analysis; however, the study results could not demonstrate the removal of other surface organic contaminants.

Our study results were consistent with the study of John et al. (2014), showing that Ti brushes were superior to other methods in the mechanical removal of bacteria and other

surfaces contaminants. This was achieved within a shorter cleaning time than curettes. However, Ti brushes caused significant change in the Ti surfaces morphology. The surface roughness was not evaluated in the current study but a previous study, using both profilometry and confocal microscopy, found that Ti brushes induce surfaces morphological changes without changing surface roughness (Park et al. 2015).

Our study results confirmed the bactericidal activity of Er: YAG laser on bacteria. However, our XPS data did not show the ablation properties claimed for Er: YAG laser, as the levels of surfaces organic contaminants were comparable before and after treatment, indicating that the inactivated bacteria and degraded biofilm were not removed from

the surface. This could be attributed to the selective absorbance of this laser energy by water in the biofilm, which causes bacterial inactivation (Matsuyama et al. 2003; Aoki et al. 2015), but it is insufficient to cause ablation of the organic molecules on the Ti surface.

The remaining degraded bacteria and biofilm on the surfaces can seed further bacterial colonization, encouraging fast reinfection by hindering the interaction of the bone cells with the surfaces and prevent re-osseointegration (Wagner et al. 2004). This could explain the reported unstable clinical improvement obtained with laser therapy (Renvert et al. 2012). The present results are in contrast with previous studies that claimed a significant reduction of plaque biofilm after cleaning with Er: YAG laser (Schwarz et al. 2005, 2006a,b). However, in these studies, the surface chemistry was not evaluated thus the surface cleanliness was not certain.

Subsequently, we can speculate that the combination of Ti brushes and laser therapy could be an effective protocol for the management of peri-implantitis in dental clinics. This protocol would involve an initial cleaning of contaminated implant surfaces with Ti brushes to remove bacteria and organic contaminants followed by Er: YAG laser treatment to disinfect the surfaces and eradicate the remaining bacteria.

One limitation of our study could be the use of Ti discs; although they have similar microstructure to implant surface, cleaning implant fixtures with screw threads is more challenging. However, the methodology used in this study has been proven to be useful for comparisons between different cleaning methods (Idlibi et al. 2013; Charalampakis et al. 2014; John et al. 2014).

Another limitation is using *in vivo* supra-gingival biofilm model instead of complex pathological biofilm that could be more resistant to decontamination. Nevertheless, our results demonstrated the inability of all the tested decontamination methods to remove the soft and less pathogenic biofilm, and thus, it could be predicted that they will also be ineffective in removing more complex biofilms.

Furthermore, in this study, live/dead bacterial assays were used to investigate the antibacterial efficacy of the tested decontamination methods on the biofilm. However, we recommend future studies to further investigate specific pathogenic bacterial species using quantitative methods such as fluorescence *in situ* hybridization or quantitative real-time polymerase chain reaction

(Colombo et al. 2013; Sánchez et al. 2013; Thurnheer & Belibasakis 2015).

Within the limitation of this *in vitro* study, we can conclude that Ti brushes were more effective than curettes and Er: YAG laser in the removal of surface contaminants, whereas Er: YAG laser was more effective than curettes and Ti brushes in killing the biofilm bacteria. None of the methods tested in this study was able to completely eliminate Ti surface contaminants.

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Conflict of interest

The authors report that there is no conflict of interest related to this study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Descriptive of the elemental composition of Ti surfaces before and after each decontamination method.

Table S2. Descriptive of the bacterial load on Ti surfaces before and after each decontamination method.

Table S3. Descriptive of the percentage change in surface chemistry due to decontamination.

Table S4. Descriptive of the percentage change in bacterial load due to decontamination.