

ORIGINAL ARTICLE

Comparison between four different implant surface debridement methods: an *in-vitro* experimental studyMagda MENSI¹, Lorenzo VIVIANI¹*, Raffaele AGOSTI¹,
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ABSTRACT

BACKGROUND: Peri-implantitis treatment is a very challenging topic to discuss. What is certain is that preventive/supportive therapy plays a key-role in peri-implant tissues' health maintenance and non-surgical implant surface mechanical debridement remains one of the solid pillars in the therapeutic pathway. In this perspective, many surface decontaminating methods have been proposed and tested to remove hard and soft bacterial deposits. The aim of this study was to compare four different commonly used non-surgical implant debridement methods in terms of cleaning potential *in vitro*, using a peri-implant pocket-simulating model.

METHODS: Sixty-four dental implants were ink-stained and placed into a simulated peri-implant pocket. Samples were then divided into four groups and treated with different debridement methods: stainless-steel ultrasonic tip (PS), peek-coated ultrasonic tip (PI), sub-gingival air-polishing with erythritol powder (EHX) and sub-gingival air-polishing with glycine powder (GLY). For each treatment group, half of the samples were treated for 5 seconds and the other half for 45 seconds. High-resolution images were taken using a digital microscope and later analyzed with a light processing software for measuring the cleaned area percentage (ink-free). Two different images were captured for every sample: a first image with the implant positioned perpendicular to the microscope lenses (90°) and a second one with the implant placed with a 45° vertical angulation, with the smooth neck towards the ground. Percentage of removed ink was statistically modelled using a generalized linear mixed model with the implant as a random (clustering) factor.

RESULTS: A paired comparison between all treatments in terms of debridement potential (cleaned area percentage) was performed. In 5s and with 90° sample angulation EHX/PS comparison showed an odds ratio of 2.75 (P<0.001), PI/EHX an OR of 0.20 (P<0.001), GLY/PS an OR of 2.90 (P<0.001), PI/GLY an OR of 0.19 (P<0.001) and PI/PS an OR of 0.56 (P=0.105). With the same sample angulation and 45s treatment time, the OR was 6.97 (P<0.001) for EHX/PS comparison, 0.14 (P<0.001) for PI/EHX comparison, 4.99 (P<0.001) for GLY/PS, 0.19 (P<0.001) for PI/GLY and 0.95 for PI/PS (P=0.989). With 5s of treatment time and 45° sample angulation, EHX/PS comparison shows a 3.19 odds ratio (P<0.001), PI/EHX a 0.14 odds ratio (P<0.001), GLY/PS a 3.06 odds ratio (P<0.001), PI/GLY a 0.15 odds ratio (P<0.001) and PI/PS a 0.46 odds ratio (P=0.017). With the same sample angulation but 45s treatment time, EHX/PS comparison produced an odds ratio of 4.90 (P<0.001), PI/EHX an OR of 0.20 (P<0.001), GLY/PS an OR of 8.74 (P<0.001), PI/GLY an OR of 0.11 (P<0.001) and PI/PS an OR 0.96 of (P=0.996).

CONCLUSIONS: Among the four treatments considered, air-polishing therapy represents the best one in terms of ink removal from the implant surface. Furthermore, increasing the treatment time to 45 seconds, air-polishing resulted considerably more efficient.

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KEY WORDS: Dental implants; Biofilms; Erythritol; Peri-implantitis.

For more than 50 years titanium dental implants had been used in dentistry for edentulous' sites rehabilitation, gradually becoming a

highly successful therapeutic option for the mid and long-term (10-years and more).¹

Researches demonstrated that osseointegrated

implants show a mean survival percentage of 96.4% in the long-term² whereas the mean success rate, according to Albrektsson *et al.* criteria, is set to 89.7% (SD of 10.2%).³

Despite these reassuring percentages, implant supporting tissues can be affected by peri-implant mucositis and peri-implantitis, with a mean prevalence of respectively 43% (95% CI: 32-54%) and 22% (95% CI: 14-30%) of the patients.⁴

Peri-implant mucositis is defined as a reversible inflammation of soft tissues surrounding an endosseous implant, without any peri-implant supporting bone loss,⁵ while peri-implantitis is defined as a non-reversible inflammatory lesion of the peri-implant soft tissues with a progressive loss of supporting bone, which can lead to biological, aesthetic and functional impairment, up to the loss of the implant.⁶

Identified risk-factors of both diseases are systemic condition of the patient, family history of chronic periodontitis, genetic traits, poor oral hygiene and lack of regular maintenance care of the implant.^{7, 8} However, the principal etiologic factor of peri-implantitis is the bacterial colonization of peri-implant tissues,⁹ followed by the development of a complex and heterogeneous oral microbiota composed of many gram-negative pathogenic species (some of them also associated with periodontitis), including *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella nigrescens*, *Prevotella intermedia*, and *Fusobacterium nucleatum*.¹⁰⁻¹²

Similarly to periodontitis therapy, because of their common etiology, peri-implantitis therapy needs to be anti-infective combining a mechanical implant surface debridement (both subgingival and supragingival) and proper education for the patient about adequate and tailor-made implant maintenance techniques.⁷

Even before the establishment of peri-implant diseases, preventive/supportive therapy plays a fundamental role in implant health maintenance. For this purpose, many debridement methods have been proposed and studied to remove hard and soft bacterial deposits with the secondary aim of not damaging nor altering the implant surface.

Plastic and metal curettes, ultrasonic devices, air-polishing systems, rubber-cups, titanium brushes and chemical decontamination

are the most commonly used prophylaxis instruments.¹³⁻¹⁵ Among them, air-polishing shows the most encouraging results *in vitro*, in terms of cleaning potential and surface unalteration.¹⁴⁻¹⁶

In particular, Quintero *et al.*¹⁷ demonstrated the superiority of air-polishing therapy over traditional ultrasonic instrumentation for implant biofilm decontamination purposes, treating biofilm colonized dental implants *in vitro*.

Furthermore, Keim *et al.* in 2019 compared three different implant surface decontamination approaches *in vitro* (air-polishing, steel curette and ultrasonic scaler), considering also multiple bone defects' configurations: air-polishing technique resulted both the most efficient and also the less damaging among them.¹⁸

In order to confirm these promising results and to directly confront different implant debridement techniques, this *in-vitro* study was conducted.

Principal objective of the present study is to compare four different commonly used non-surgical implant debridement methods in terms of cleaning potential *in vitro*, using a peri-implant pocket-simulating model.

Our hypothesis is the superiority of the air-polishing techniques in removing ink-simulated biofilm over traditional ultrasonic instrumentation.

Materials and methods

In-vitro model preparation

This study was performed using 64 dental implants with a diameter of 3.8 mm and a length of 11.5 mm (Premium Kohno®; Sweden & Martina, Due Carrare, Padua, Italy). The implants were completely coated *via* immersion in a non-soluble and permanent black ink (Staedler permanent Lumocolor® black, Nürnberg, Germany), as a visual simulation of the biofilm surface colonization.^{16, 19} After ten minutes of air-drying, the implants were inserted in a semi-circular vertical simulated defect with a length of 10 mm and a diameter of 3.8 mm, carved into a stainless steel rectangular block. A second custom-made and carved metal block provided with a silicon covering was applied to the first in correspondence of the defect. These two pieces were held to-

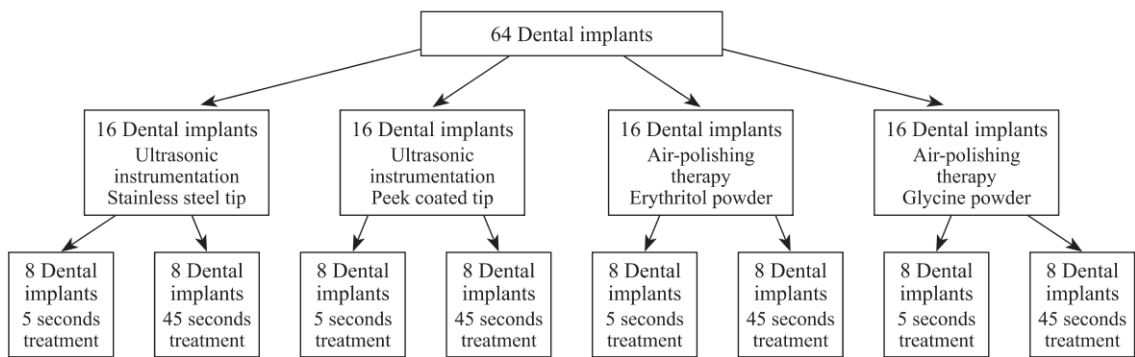


Figure 1.—Study design.

gether in position by a spring clamp. The silicon material worked as a peri-implant soft tissue replacement to better simulate visual and tactile in vivo working conditions, and it allowed a stable fixation of the implant during treatments.

Simulated treatments

The implants were divided into four groups and treated with different surface instrumentation:

- a stainless-steel metal tip [PS tip®, EMS, Nyon, Switzerland] mounted on an ultrasonic device (AirFlow Prophylaxys Master®, EMS, Nyon Switzerland), used at 50% power setting and 100% irrigation setting;
- a PEEK coated tip (PI tip®, EMS, Nyon, Switzerland) mounted on an ultrasonic device (AirFlow Prophylaxys Master®, EMS, Nyon Switzerland), used at 70% power setting and 100% irrigation setting;
- an air-polishing device (AirFlow Prophylaxys Master®, EMS, Nyon, Switzerland) using glycine powder (Perio® powder, EMS, Nyon Switzerland) conveyed by a nozzle designed for subgingival use (Perioflow®, EMS, Nyon, Swit-

zerland) at 100% power setting and 100% irrigation setting;

- an air-polishing device (AirFlow Prophylaxys Master®, EMS, Nyon, Switzerland) using erythritol powder (Plus® powder, EMS, Nyon Switzerland) conveyed by a nozzle designed for subgingival use (Perioflow®, EMS, Nyon, Switzerland) at 100% power setting and 100% irrigation setting.

All treatments were performed by the same operator (an expert dentist) for 5 and 45 seconds. Eight dental implants were randomly assigned for each of the eight total groups (Figure 1).

Timed and controlled treatments were conducted with constant movements, both up-down and left-right, to better simulate the real clinical use (Figure 2).

Implants were then extracted from the model, water-sprayed for 10 seconds to remove powder or ink debris and air-dried.

Measurements of cleaned surface percentages

High-resolution images of the implant surface were taken by the same operator using a digital

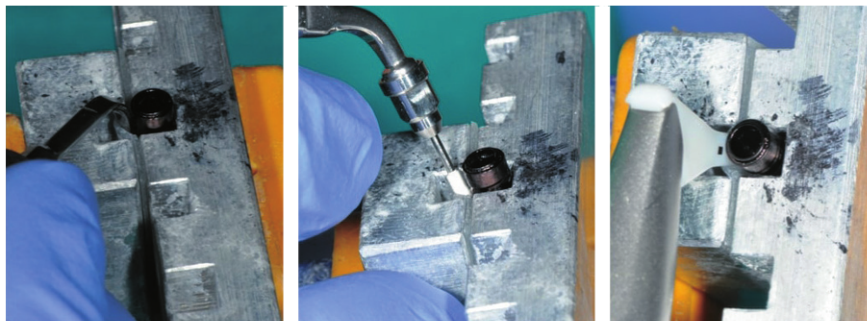


Figure 2.—Simulated treatments performed under controlled and stopwatch-timed conditions: from left to right, ultrasonic instrumentation with stainless steel and PEEK-coated tip, subgingival air-polishing therapy with erythritol powder.

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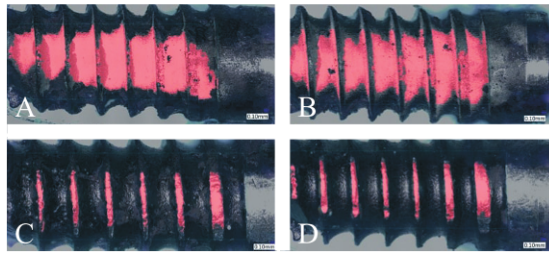


Figure 3.—A sample of the images obtained *via* 90° acquisition. Implants treated for 45 seconds with subgingival air-polishing with Erythritol powder (A) and Glycine powder (B); implants treated for 45 seconds with ultrasonic instrumentation with stainless steel (C) and PEEK-coated tip (D).

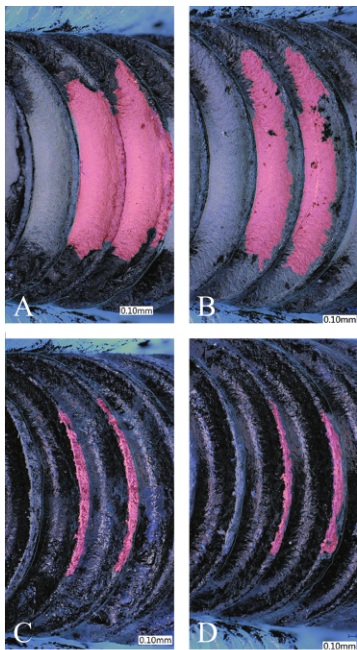


Figure 4.—A sample of the images obtained *via* 45° acquisition. Implants treated for 45 seconds with subgingival air-polishing with Erythritol powder (A) and Glycine powder (B); implants treated for 45 seconds with ultrasonic instrumentation with stainless steel (C) and PEEK-coated tip (D).

microscope (VHX-6000 Digital Microscope®, Keyence Corporation, Osaka, Japan) with standardized settings. Two different of images were acquired for every sample treated to assess the cleaning potential both over and under implant threads: a first image with the implant positioned perpendicularly to the microscope lenses (90°) and a second one with the implant positioned with a 45° vertical angulation, with the smooth neck towards the ground. A resin custom made

frame was used to hold in position the sample during the image acquisition.

Every image was then analyzed using the light processing software of the microscope in order to assess the percentage of the cleaned area. For the 90° images, a rectangular area of 7.00 mm length and 2.50 mm width (17.50 mm² total area) was used (Figure 3). For the 45° images, a manual selection of the third and fourth thread surface was performed (about 5.00 mm² total area) and then analyzed (Figure 4).

Statistical analysis

The percentage of plaque removed was modelled using a Generalized Linear Mixed Model with the implant as a random (clustering) factor. Due to the nature of the outcome variable (a proportion), we assumed a Beta distribution for the errors with logit link.

Results

For the sake of simplicity, treatment groups will be named as listed below:

- EHX: Air-polishing with erythritol powder conveyed by sub-gingival nozzle;
- GLY: Air-polishing with glycine Powder conveyed by sub-gingival nozzle;
- PS: Ultrasonic debridement with PS Tip®;
- PI: Ultrasonic debridement with PI Tip®.

The data obtained by digital assessment of the cleaned implant surface (percentage of removed ink) show us that a complete (100%) ink removal from the analyzed zone was impossible and that the maximum ink removal percentage (62.78% and 58.57%, respectively at 90° and 45° angulation) was achieved by subgingival erythritol air-polishing.

Analyzing the images taken with the 90° angulation (Figure 5), EHX obtained an average percentage of ink removal of 17.39±4% with 5s treatment time and an average percentage of ink removal of 52.67±6% with 45s treatment time; on the other hand, GLY obtained an average percentage of 18.43±5% at 5s and 44.39±9% at 45s; PS achieved an average 6.61±1% with 5s timing and 13.38±2% with 45s timing, whereas PI resulted in 3.74±1% ink removed at 5s and 13.69±1% at 45s. When the 45° angulation im-

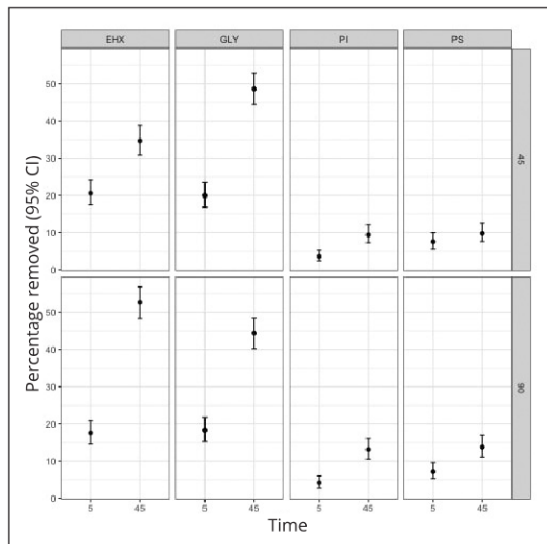


Figure 5. Data representation with confidence intervals values. The graph displays on the X-axis the treatment time and on the Y-axis the mean percentage of removed ink. Vertical divisions of the graph are determined by the four different treatments confronted (EHX, GLY, PI and PS) and horizontal divisions by the sample angulation (45° and 90°).

ages were analyzed, EHx showed an average ink removal percentage of 20.75±5% in 5s treatment time and 35.10±12% in 45s treatment time, while GLY obtained a 20.06±6% with 5s treatment time and 49.05±8% with 45s treatment time. With a treatment time of 5s, PS and PI instrumentation resulted in 7.05±2% and 3.37±2% ink removal respectively; with 45s timing, the same instrumentations resulted in 9.51±3% and 9.21±4% ink removal respectively.

In Table I a pairwise comparison between all treatments was performed, grouping each treatment for image acquisition angulation and treatment time. In 5s and with 90° sample angulation EHx/PS comparison showed an odds ratio of 2.75 (P<0.001), PI/EHx an OR of 0.20 (P<0.001), GLY/PS an OR of 2.90 (P<0.001), PI/GLY an OR of 0.19 (P<0.001) and PI/PS an OR of 0.56 (P=0.105). With the same sample angulation and 45s treatment time, the OR was 6.97 (P<0.001) for EHx/PS comparison, 0.14 (P<0.001) for PI/EHx comparison, 4.99 (P<0.001) for GLY/PS, 0.19 (P<0.001) for PI/GLY and 0.95 for PI/PS (P=0.989). With 5s of treatment time and 45° sample angulation, EHx/PS comparison shows a 3.19 odds ratio (P<0.001), PI/EHx a 0.14

odds ratio (P<0.001), GLY/PS a 3.06 odds ratio (P<0.001), PI/GLY a 0.15 odds ratio (P<0.001) and PI/PS a 0.46 odds ratio (P=0.017). With the same sample angulation but 45s treatment time, EHx/PS comparison produced an odds ratio of 4.90 (P<0.001), PI/EHx an OR of 0.20 (P<0.001), GLY/PS an OR of 8.74 (P<0.001), PI/GLY an OR of 0.11 (P<0.001) and PI/PS an OR 0.96 of (P=0.996).

Data resulting from pairwise comparisons between the two air-polishing treatments (EHx/GLY) are displayed in Table II. OR resulting from the EHx/GLY comparison in 5 s of treat-

TABLE I.—Pairwise comparison between all four treatments grouped by sample angulation (45° and 90°) and treatment time (5 and 45 seconds).

Treatment time	Sample angulation	Comparison	Odds ratio	P value
5s	45°	PI/EHx	0.14	<0.001
		PI/GLY	0.15	<0.001
		PI/PS	0.46	0.017
		EHx/GLY	1.04	0.993
		EHx/PS	3.19	<0.001
		GLY/PS	3.06	<0.001
	90°	PI/EHx	0.20	<0.001
		PI/GLY	0.19	<0.001
		PI/PS	0.56	0.105
		EHx/GLY	0.95	0.985
		EHx/PS	2.75	<0.001
		GLY/PS	2.90	<0.001
45s	45°	PI/EHx	0.20	<0.001
		PI/GLY	0.11	<0.001
		PI/PS	0.96	0.996
		EHx/GLY	0.56	<0.001
		EHx/PS	4.90	<0.001
		GLY/PS	8.74	<0.001
	90°	PI/EHx	0.14	<0.001
		PI/GLY	0.19	<0.001
		PI/PS	0.95	0.989
		EHx/GLY	1.40	0.033
		EHx/PS	6.97	<0.001
		GLY/PS	4.99	<0.001

TABLE II.—Pairwise comparison between air-polishing treatments with Erythritol (EHx) and Glycine (GLY) powders, grouped by sample angulation (45° and 90°) and treatment time (5 and 45 seconds).

Treatment time	Sample angulation	Comparison	Odds ratio	P value
5s	45°	EHx/GLY	1.04	0.7871
	90°	EHx/GLY	0.95	0.7241
45s	45°	EHx/GLY	0.56	<0.001
	90°	EHx/GLY	1.40	0.0067

TABLE III.—Pairwise comparison between air-polishing treatments with erythritol (EHX) and glycine (GLY) powders and ultrasonic instrumentations with peek coated tip (PI) and stainless-steel tip (PS), grouped by sample angulation (45° and 90°) and treatment time (5 and 45 seconds).

Treatment time	Sample angulation	Comparison	Odds ratio	P value
5s	45°	EHX-GLY/PI-PS	4.50	<0.001
	90°	EHX-GLY/PI-PS	3.68	<0.001
45s	45°	EHX-GLY/PI-PS	6.55	<0.001
	90°	EHX-GLY/PI-PS	5.97	<0.001

TABLE IV.—Pairwise comparison between sample angulations (90° and 45°) in air-polishing treatments with Erythritol (EHX) and Glycine powder (GLY) with 45 seconds timing.

Treatment	Comparison	Odds ratio	P value
EHX	90°/45°	2.10	<0.001
GLY	90°/45°	0.84	0.16

ment time were 1.04 (P=0.7871) for the 45° sample angulation, and 0.95 (P=0.7241), for 90° sample angulation. Both results were not statistically significant.

On the other hand OR resulting from the EHX/GLY comparison with 45s timing were 0.56 (P<0.001) with 45° sample angulation, and 1.40 (P=0.0067) with 90° sample angulation. Only the 45° comparison was statistically significant.

Table III presents the data regarding the comparisons between the two air-polishing treatments (EHX and GLY) and ultrasonic instrumentation (PI and PS): while with 5s treatment time the OR was 4.50 (P<0.001) and 3.68 (P<0.001), for 45° and 90° sample angulation respectively, with 45s treatment time the OR resulted in 6.55 (P<0.001) and 5.97 (P<0.001), for 45° and 90° sample angulation respectively.

Data resulting from the comparison between the two acquisition angles (90°/45°) are displayed in Table IV: EHX treatment resulted in a 2.10 OR (P<0.001), while GLY treatment resulted in a 0.84 OR (P<0.16), therefore not statistically significant.

Discussion

The objective of this *in-vitro* study was to assess *via* controlled *in-vitro* conditions the best

debridement method among the four examined, in terms of percentage of ink removed from the micro-rough implant surface. Chosen implant surface was a sandblasted and acid-etched surface: this type of implant surface is currently regarded as the most predictable and reliable one, from a clinical, microbiological, histological and biomechanical point of view.²⁰ The data obtained confirmed our hypothesis about the cleaning superiority of air-abrasive devices (using erythritol or glycine powder) over ultrasonic-driven instrumentation (using a stainless-steel tip or a PEEK coated one). These results have been confirmed with different treatment timings (both 5 and 45 seconds) and angulation of sample analysis (both 90° and 45°). To date, there is no stated and clean therapeutic strategy to treat peri-implant diseases.^{15, 21} Surely, according to literature, a non-surgical debridement therapy (manual, ultrasonic driven or air-abrasive) should always be used to remove biofilm and reduce bacterial load on implant structures as much as possible before any surgical intervention.²² Moreover, peri-implantitis defects show a non-predictable response to both surgical and non-surgical therapy²² and surgical therapies presented no better clinical outcomes than non-surgical therapies. For these reasons, non-surgical implant debridement appears to be the best treatment option now clinically available.²³ In these terms, among all debridement methods available, air-polishing therapy showed promising results *in vitro*,^{21, 24-26} thanks to its cleaning potential and its harmlessness towards implant micro and macro-structure. Nevertheless, the lack of scientific evidence for implant subgingival air-polishing debridement and comparison of different powders (*i.e.* erythritol or glycine) under simulated non-surgical approach conditions, made necessary to perform further studies to investigate its real cleaning potential. For this reason, the authors decided to conduct the present *in-vitro* study. As shown by our results, EHX and GLY showed a clear superiority compared to PI and PS, regardless of the treatment time and angulation of analysis. The difference between air-abrasive and ultrasonic driven instrumentation appears more marked when increasing the treatment time. In fact, when treating the sample for 5s, the aver-

age difference in terms of removed ink is around 13-15% in favor of air-abrasive instrumentation for 90° images and 45° images respectively, while with 45 seconds of treatment this difference is around 33-35%. As we can see from the 90° digital images collected, the application of air-polishing provided a wider and more spread clean area on the implant surface. In addition, it could remove ink both from the top and in-between of the threads considered. When observing the 45° digital images, it clearly appears how air-polishing is able to widely clean the semi-circular area immediately below threads as well as the top of threads, with both powders tested. On the other hand, ultrasonic instrumentation shows an inferior debridement potential, with incomplete cleaning areas and only limited to the top of the threads. These results can be easily understood observing the shape of the cleaning instruments: straight and rigid tips (both metal or plastic-coated) unable to reach areas between or under the threads, while air-blasted micron-sized powders particles can clean thanks to their high-speed free-movement and physical interactions. In regard to the comparison between EHX and GLY, at this stage is not possible to draw definitive conclusions. Based on our results, while with 5s of treatment time the average percentage of removed ink is comparable at between the two powders, with 45s of treatment time, EHX resulted more effective (52.67±6%) than GLY (44.39±9%) at 90° sample angulation but, at 45° sample angulation, GLY resulted more effective (49.05±8%) than EHX (35.10±12%). Furthermore, results obtained by pairwise comparison between the two powders are statistically significant only in the 45° images analysis for 45s treatment time. The results highlight the necessity of further studies with a different sample size to attest the cleaning superiority of one powder over the other. We assume that the differences could be due to the different powder particle size (14 µm for EHX, 25 µm for GLY) and their consequent physical interaction within the peri-implant defect model. With 5 seconds of treatment time, EHX and GLY resulted respectively in an average ink removal percentage of 17.39±4% and 18.43±5% with 90° sample angulation, 20.75±5% and 20.06±6% with 45° sam-

ple angulation. Keeping in mind the purpose of removing implant biofilm at maximum in order to obtain an improvement of the patients' clinical conditions or to maintain peri-implant tissues health, the percentage of ink removal achieved seems insufficient and not adequate. This consideration is also motivated by the fact that the implant area considered for analysis in the present study is easily accessible in the model by prophylaxis instrumentations, so a complete ink removal was expected and hoped for. Nevertheless, the authors find important to have a critical look into others *in-vitro* studies which employed the same ink (*Staedler permanent Lumocolor® black, Staedler, Nürnberg, Germany*): both Sahrmann *et al.*^{14, 19} and Ronay *et al.*¹⁶ could not obtain a complete ink removal from the implants treated in their *in-vitro* study. Among these studies, a nozzle specifically designed for the subgingival air-polishing was only employed in the study of Ronay *et al.*,¹⁶ achieving a 40.15±10.40 residual ink on the implant surface (59.85% of the cleaned area) after a 120 seconds-long treatment. The data seems perfectly aligned with our results, which show a maximum ink removal of 62.78% (EHX, 45 seconds, 90° sample angulation). Moreover, based on the "total inflammatory burden theory"²⁷, reducing biofilm of 20% could still be sufficient to completely restore the balance between colonizing bacteria and immune system cells, leading to a peri-implant mucositis condition resolution, a peri-implantitis condition arrest or peri-implant tissues health maintenance. Furthermore, the choice of employing a 5 seconds treatment time arise from the indications given by the powders' producer company (PLUS® and PERIO® powder, EMS, Nyon, Switzerland) to avoid unwanted complications such as subcutaneous emphysema. We suggest the clinical possibility of alternate 5 seconds of treatment with 5 seconds of pause during implant or tooth subgingival debridement, to safely increase total implant debridement time without raising the risk of complications. With 45 seconds of treatment time, the mean percentage of ink removed via air-polishing increases up to six/seven times (90° and 45° sample angulation, respectively) the one obtained with ultrasonic instrumentation. The percentage of ink

removed via air-polishing (up to 62.78%), could represent a clinically significant outcome during peri-implant diseases therapy and could be more than sufficient to arrest or prevent peri-implant pathology development. However, further studies are needed to improve or integrate the results obtained and to correlate these data with *in-vivo* clinical outcomes.

Limitations of the study

The difference between real and simulated peri-implant tissues, between implant colonizing biofilm and the ink used, non-perfect reproducibility of the manual instrumentation for every sample treated and the limited resolution power of the microscope were the principal technical limitations of this experimental study.

Conclusions

Nevertheless, we can conclude that, among the four treatments considered and studied, air-polishing represents the best one in terms of ink removal from the implant surface. Furthermore, increasing the treatment time from 5 seconds to 45 seconds, air-polishing devices resulted in considerably higher efficiency in simulated-biofilm removal.

However, it remains unclear which powder between erythritol and glycine holds the best cleaning potential and can be recommended for implant surface debridement.

Further *in-vitro* studies with more accurate methods and bigger sample size are needed to formulate a validated implant instrumentation protocol to be tested via randomized clinical trials and clinical observational studies.

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