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Restorative aesthetic materials: *in vitro* analysis on bacterial adhesion and iatrogenic damage induced by professional hygiene techniques

Loris Prosper*, Irene Setaro**, Elisabetta Polizzi***, Nicolas Zunica****, Clara Cassinelli*****, Carlo Alberto Cortella******

Purpose: *The objective of this* in vitro *study is to evaluate whether the restorative esthetic materials under consideration show structural surface differences that can induce and promote bacterial adhesion. In addition, on zirconia oxide samples, an attempt was made to identify a professional instrumental hygiene technique that can be defined as the preferred technique in the removal of bacterial plaque.* **Materials and methods**: *30 sample disks were produced by the DentalWork dental laboratory in Monza, divided into three groups: (1) 10 zirconia sample disks identified in Group A; (2) 10 feldspar ceramic sample disks identified in Group B; (3) 10 sample disks of composite resins identified in Group C. All 30 samples were contaminated with* Streptococcus Mutans. **Conclusions**: *from this study, the authors agree in hypothesizing that the restorative aesthetic material found to be less retentive to bacterial adhesion and replication is that of Group B (ceramics). From the acquisition of morpho-structural images performed at the SEM (Scanning Electron Microscope) it can be deduced that the professional treatment for bacterial breakdown and removal that turned out to be the preferred treatment for zirconium oxide is Air Polish Perio Soft - EMS. Hence the importance of the role of dental hygienists in identifying a professional hygiene technique to maintain prosthetic artefacts in the medium and long term, monitoring the health parameters of the oral cavity with a support plan.*

Keywords: *Bacterial plaque,* Streptococcus Mutans, *Zirconia, Glycine.*

INTRODUCTION

The new horizons in dentistry have allowed an increasingly innovative and refined choice of restorative aesthetic materials1-3. The maintenance of the fixed prosthetic fabric, in addition to allowing correct chewing function, also requires stability from the point of view of the aesthetic result in the medium and long term since the patient's expectations are now very high. Maintain tissue health over time in contact with the prosthetic restoration is not always predictable because incorrect instrumental hygiene techniques could alter the integrity and precision of the marginal closure, create retaining asperities of the plaque and thus induce iatrogenic damage to the prosthetic artifact[4-7].

Dental hygienists are directly called upon to contribute to biological success by controlling the infectious and inflammatory potential of the oral cavity8. Lo- ro, through the programming of personalized recall sessions, follow the control and evaluation of oral hygiene conditions, identifying the early signs of inflammation, in order to optimize the maneuvers of choice of IOD9-11.

Studies show that the use of the innovative gel (HOBAGEL) by HOBAMA, composed of a mix of substantive and antibacterial substances (including Cetylpyridinium, Chloride, Triclosane and essential oils) released in the context of the gel itself from microcapsules of innovative technology that greatly decreases the periodontal indices of plaque and bleeding. Its medium-low (+-3o) R.D.A. is the least abrasive on restorative aesthetic materials.[12] The intervention of the dental hygienist must be aimed at reducing the amount of plaque in correspondence with the prosthetic artefacts, directing the microbiological pool towards non-pathogenic species, so as to

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intercept any anomalies immediately and intervene promptly. The patient will be explained the home hygiene maneuvers and professional hygiene interventions will be planned in order to monitor the state of health and the integrity of the margins of the aesthetic crowns (Figs. 1a, b; 2a-c; 3).

The study was performed *in vitro* on 30 sample disks and aims to compare the bacterial adhesiveness of the surfaces of the restorative aesthetic materials that are structurally morphologically different, identifying the least retentive. The other objective is to hypothesize an instrumental technique of professional hygiene of choice in the removal of the bacterial organic matrix on prosthetic artifacts. The results of the study and the assessments of iatrogenic damage induced by the three occupational hygiene techniques (Peek tip, Air Polishing Perio soft - EMS, NUPRO paste added cup) were examined at the SEM.

All the steps of the experimental methodologies are iconographically documented.

MATERIALS AND METHODS

30 sample discs measuring 0.5 cm in diameter by 3 mm thick were produced, supplied by the Dentalwork dental laboratory in Monza. The samples divided into three groups were composed as follows:

- *Group A*: 10 zirconia discs.
- *Group B*: 10 feldspar ceramic discs.
- *Group C*: 10 composite resin discs.

All samples were sent to the Tumor Immunology laboratory of the Vita-Salute San Raffaele University for bacterial contamination with *Streptococcus Mutans*. The choice of *Streptococcus Mutans* is dictated by the fact that it is the major pathogenic candidate dentin enamel and in the formation of secondary caries .

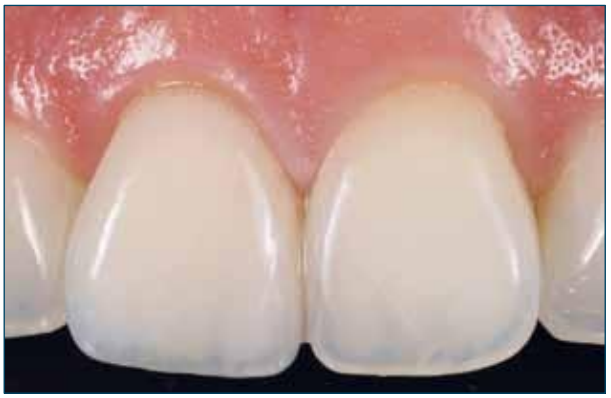
The laboratory set up the control procedure as follows :

- pre-inoculation of bacteria to make them grow 24 hours before the experiment;
- dilution of the bacterial suspension 1:10 in PBS (Phosphate Buffered Saline);
- washing of the disks in ultrapure water;
- incubation of the diskettes with the suspension of batteries for 4 hours;
- washing of the PBS disks for 10 minutes (to simulate self-cleansing of saliva).

Only group A was then divided into three subgroups + a control case and subjected to the following occupational hygiene treatments carried out under standard operator-dependent conditions (Figs. 4-6).

The disks, after undergoing professional treatment, under standard operator-dependent conditions, were sent back to the tumor immunology laboratory of the Vita-Salute San Raffaele University and subjected to the following protocol:

- Fixing of bacteria with formalin;
- staining of bacteria with Acrydine Orange solution to allow bacterial count;
- wash 2 times for 5 minutes to remove excess dye;
- visualization under fluorescence microscope (10X objective) and image acquisition (two fields per disk);
- washing of the pads in ultra-pure water for 3 hours;
- Immersion of the disks in solution: 25% absolute ethanol 75% water.



Figs. 1a,b Veneer on 11; incongruous hygiene manoeuvres or the use of improper materials risk irreparably damaging the veneers, which are imperceptible especially in the cervical areas, as demonstrated by the use of ultrasound, which in addition to chipping the closures, can roughen the transition areas between the ceramic and the natural tooth (ceramics made by Dr. Loris Prosper with Ceramica Shofu - Kyoto, Japan).

Bacterial count

The visualization of the bacterial count was carried out under a fluorescence microscope at 10X magnification using the IMAGE J (NIH) software via cell counter. The count values for two fields of each sample were then estimated, thus estimating the cell count result.

Subsequently, only the Group A diskettes were sent for morphological and structural analysis to the Nobil Bio Ricerche laboratory in Portacomaro (AT).

The protocol performed by the SEM is set out below:

- The samples were dehydrated with water and ethanol in successive concentrations:
  - water 75% - ethanol 25%,
  - water 50% - ethanol 50%,
  - water 25% - ethanol 75%,
  - 100% ethanol.

In a second step, the samples were metallized using the Agar Sputter Coater (Agar Scientific) with a gold sheet (99.9% purity) and mounted on a sample holder with conductive carbon-based double-sided adhesive tape and observed with SEM EVO-MA10 (Zeiss - Germany).

Figs. 2a-c Ceramic veneers of 11-21-12-22 where the concealment of the artifacts that can deceive the hygienist or the operator who implements the maneuvers for the removal of bacterial plaque is highlighted, thus creating iatrogenic and irreparable damage to the prosthetic artifact (ceramics made by Dr. Loris Prosper with Ceramica Shofu - Kyoto, Japan).

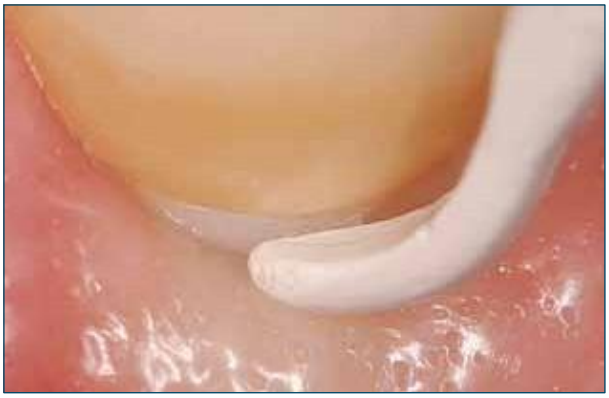


Fig. 3 Zirconium abutment and all-ceramic crown. By inspecting the sulcus, it is noted that these materials maintain periodontal integrity. By detaching the groove with a Hu-Friedy Teflon courette, you can see the precision of the closure of the prosthetic artifact with its abutment, a fundamental and basic principle for the preservation and respect of the free marginal gingiva (ceramics made by Dr. Loris Prosper with Ceramica Shofu - Kyoto, Japan).

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Fig. 4 The samples of Group A1 were treated with a Peek tip (EMS) with a 5" time protocol with horizontal movements.

Fig. 5 The samples of Group A2 were treated with the Air Polishing (EMS) technique with the addition of Perio Soft with a 5" protocol at a distance of 5-3 mm from the disk with an inclination of 30°/60°.

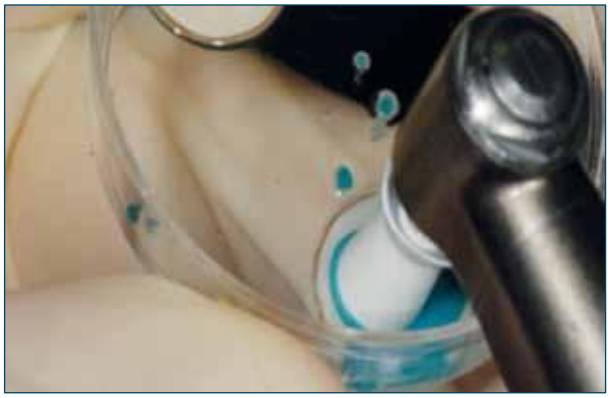


Fig. 6 The samples of Group A3 were treated with rubber cups and fine-grained NUPRO polish paste with a 5" time protocol.

- The images were taken at different magnifications:
  - 300X,
  - 1000X,
  - 3000X,
  - 5000X,
  - 10000X,

  in different areas in each of the two replication replicas .
- The salient parameters of the analysis are shown in the strip at the bottom of each photo:
  - Electron acceleration potential = EHT.
  - Focal Distance = WD.
  - Magnification = MAG.
  - Type of detector used = SIGNAL A.
  - Date.
  - Progressive number.
  - Dimensional parameter .

RESULTS

Microbiological

The bacteria were examined under a fluorescence microscope at 10X after being stained with Acrydine Orange solution to estimate the bacterial count. All groups (A, B and C) were compared: the average of the bacterial con- tes of each material under the 10X fluorescence microscope allowed to identify the number of replicas of the colonies on the surfaces: the result was that the surfaces that retain less plaque were ceramics (Group B), followed by zirconia (Group A) and composite resins (Group C) (Fig. 7; Table 1).

The results of Group A show that:

- *Group A1*: the most effective treatment in the removal of bacterial colonies was the Air Polish (EMS) technique with Perio Soft powder (Table 2).
- *Group A2*: the polishing technique with mechanical instrumentation with the aid of prophylaxis paste and cup was found to be effective only in the contact/ treatment areas. Therefore a partial removal.

Fig. 7 Acquisition of a 10X fluorescence microscope image.

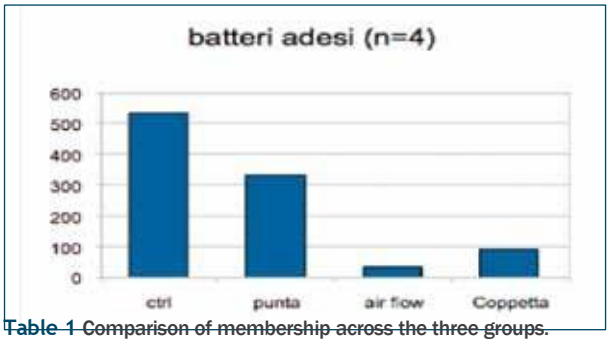


Table 1 Comparison of membership across the three groups.

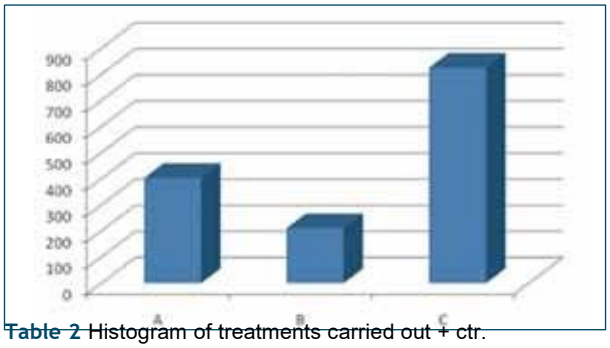


Table 2 Histogram of treatments carried out + ctr.

- *Group A3*: The Peek tip has broken down and disas-shaped the bacterial colonies, but has not been effective in complete removal.

The histogram graph consists of the treatments performed plus the case-control on the x-axis and the number of bacterial counts on the y-axis. It is evident that the treatment performed with Air Polish perio (EMS) is the result of choice.

Morphological

From the acquisitions of the images analyzed at the SEM on Group A, it emerged that:

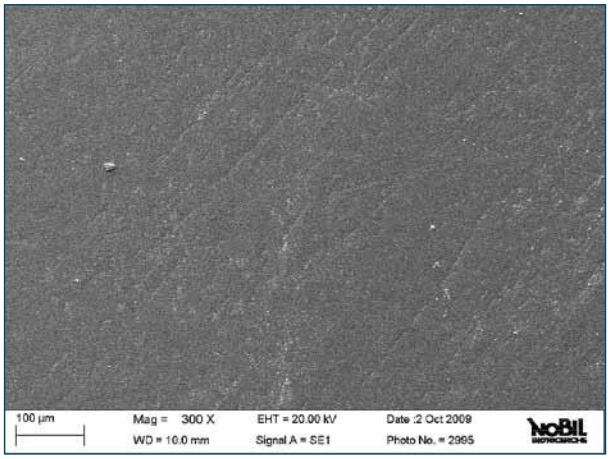
- group A in the case of zirconia control has surface irregularities already at 300X; this is due to the structural characteristic of zirconium oxide and the polishing treatment carried out by the laboratory (Figs. 8-11).
- Untreated inoculated zirconia has bacterial colonies that can be seen concentrated "in cushions" dispersed over the entire surface already at 1000X (Figs. 12-14).
- *Group A1* (Peek tip): does not remove colonies, but breaks them up causing clear "scratches" or "furrows" already evident at 300X (Fig. 15).
- *Group A2* (Air Polish Perio - EMS): there is a total elimination of bacterial colonies with glycine residues on the treated surface (Figs. 16-18).
- *Group A3* (cup): the surface treated with the cup has residues of prophylaxis paste and the removal of *Streptococcocus Mutans* colonies is only in the areas of contact with the device (Figs. 19-22).

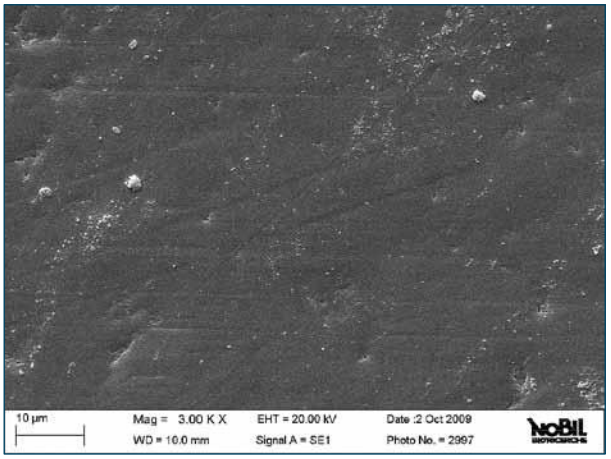
CONCLUSIONS

Prosthetic rehabilitation on both natural teeth and implants is a fundamental step in restoring chewing function. The patient's quality of life and aesthetic expectations are high and this is the reason why the clinician/prosthetist resorts to the need to use increasingly sophisticated aesthetic materials in the construction of the prosthetic product.

It is therefore important in any rehabilitation process that the operator should keep and preserve it.

Hence the need for the dental hygienist to formulate a long-term support plan that is effective





Figs. 8,9 Structural irregularities of non-inoculated zirconia.

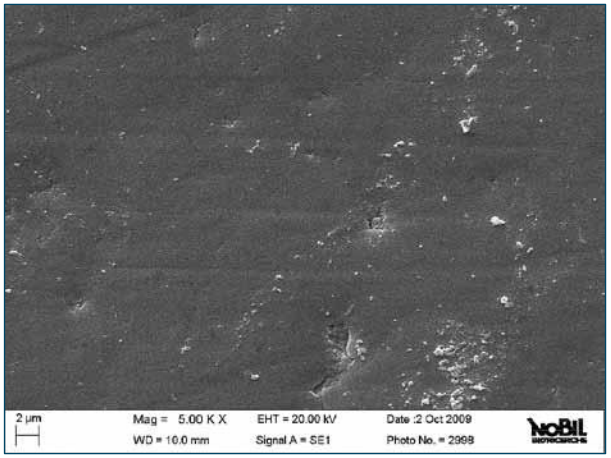


Fig. 10 Zirconia surface at 5000X.

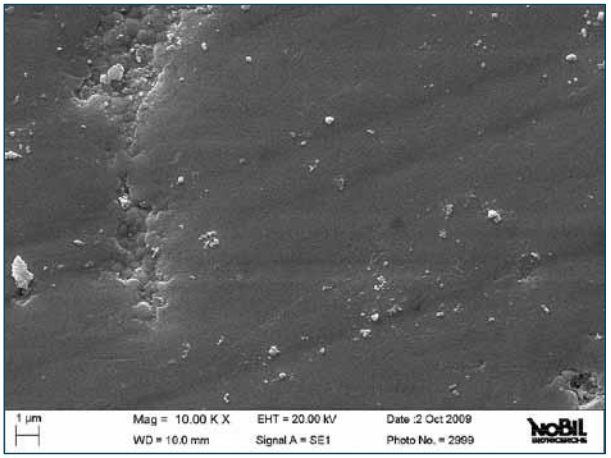


Fig. 11 Presence of roughness on the surface due to the morphostructure of zirconia and the treatments performed by the laboratory for sample preparation.

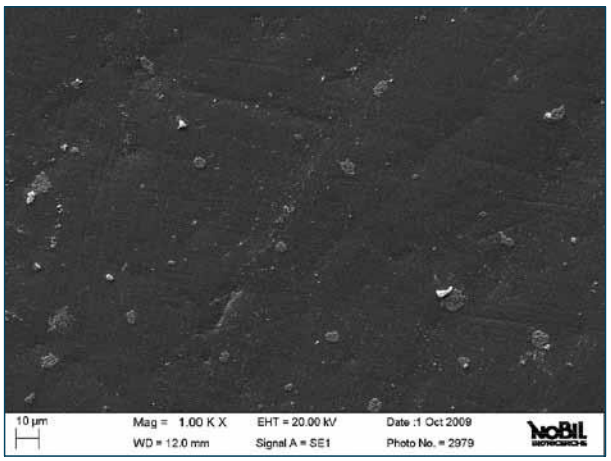


Fig. 12 Colonies dispersed on the surface.

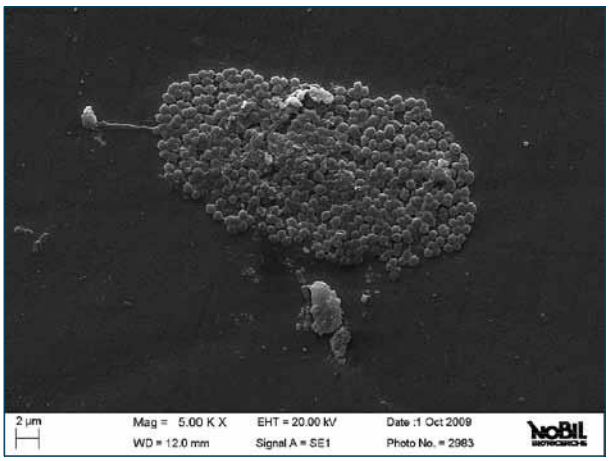


Fig. 13 Cluster in a colony of *Streptococcus Mutans* at 5000X.

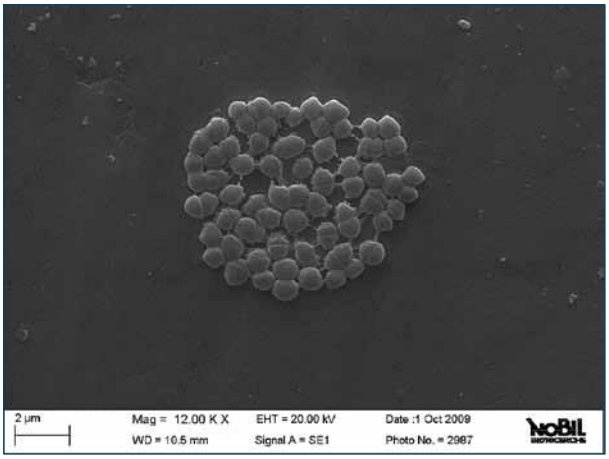


Fig. 14 Detail of a colony of *Streptococcus Mutans* at 12000X.

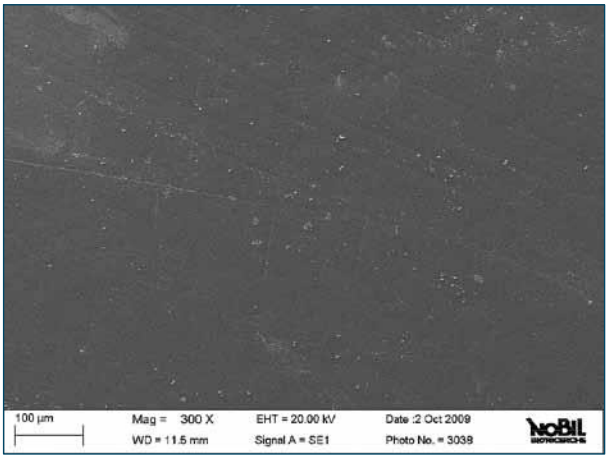


Fig. 15 Presence of scratches on the surface.

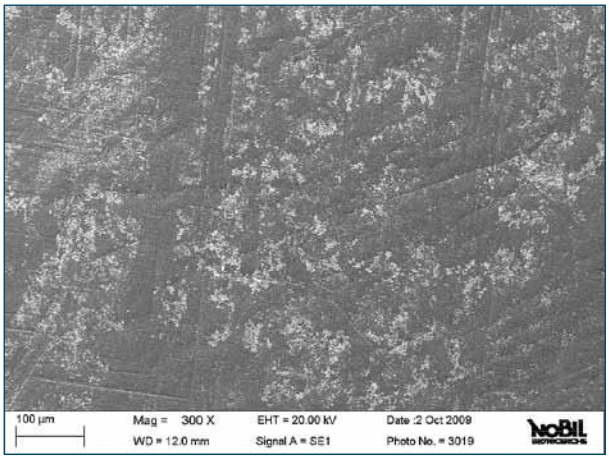


Fig. 16 Total removal of bacterial colonies visible already at 300X.

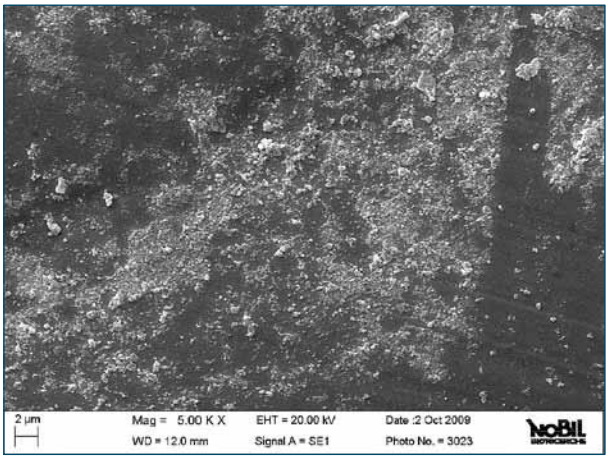


Fig. 17 Image captured at 5000X.

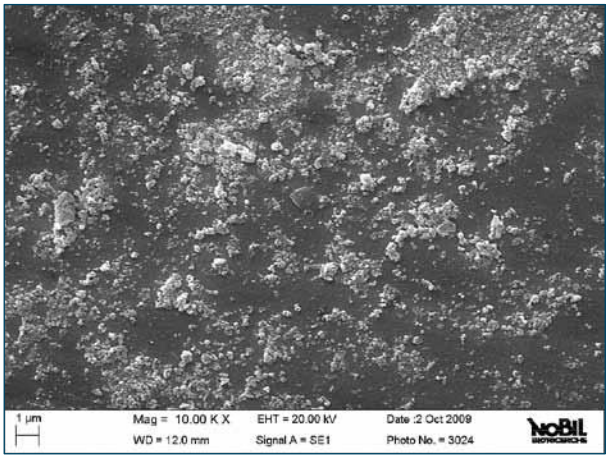


Fig. 18 Absence of bacterial colonies on the surface; glycine powder residues are found.

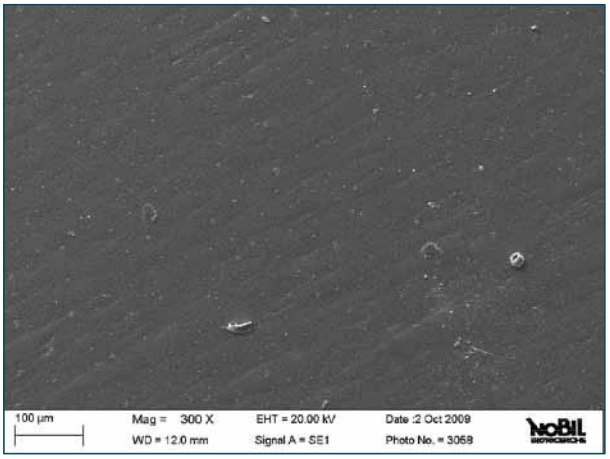


Fig. 19 Presence of bacterial colonies visible at 300X.

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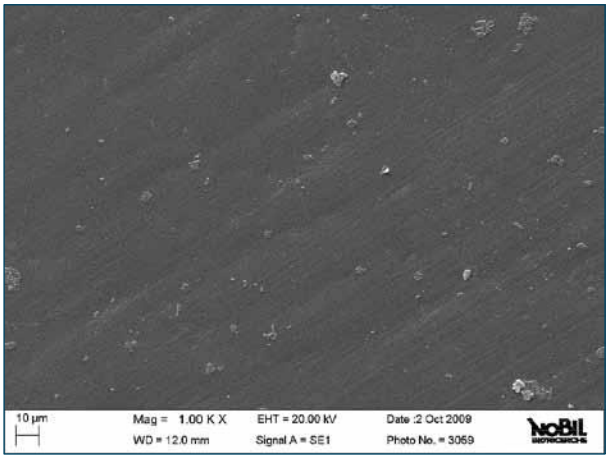

Fig. 20 1000X image capture.

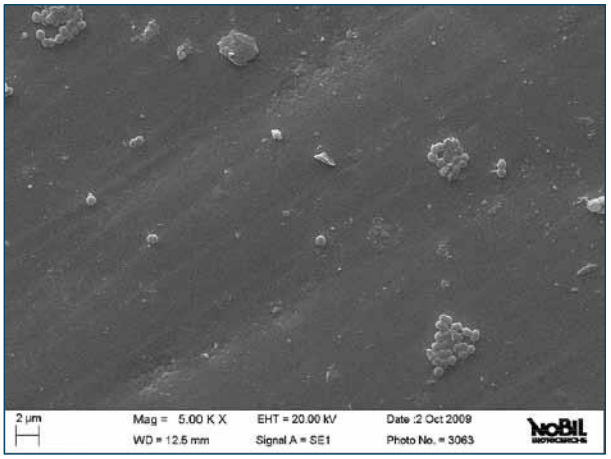

Fig. 21 Colony removal is only done at the point of contact/treatment with the device.

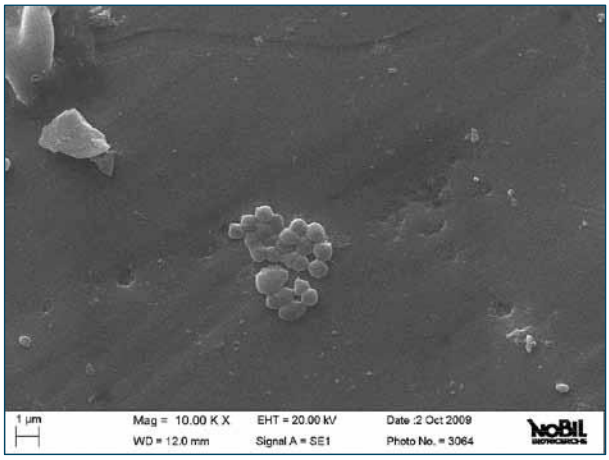

Fig. 22
Colony of *Mutans Streptococci* at 10000X.

in maintaining a high level of patient attention as well as compliance, so that professional oral hygiene sessions can be punctuated and all health parameters under control and thus maintain the prosthetic products in the long term without causing iatrogenic damage.

In the light of the results obtained, the authors agree that the treatment of choice for the removal of bacterial colonies is Air Polish (EMS) with Perio Soft glycine powder; however, it should be considered that the study was performed on a small number of samples, therefore the specific analyses on the bacterial adhesiveness to these restorative aesthetic materials would deserve further investigation.

The acquisition of images and SEM analyses show that glycine powder is, therefore, eli- totally undermine the colonies of *Streptococcus Mutans* without causing alterations to the surface of zirconium oxide. On the other hand, treatments performed with a Polish cup with NUPRO prophylaxis paste and a Peek tip are partially or totally ineffective. It can be seen from observations that the latter visibly damages the surface of the zirconium oxide sample disks.

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