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Evaluation of dog tooth enamel by scanning electron microscopy after different types of polishing

Avaliação do esmalte dentário de cão por microscopia eletrônica de varredura após diferentes tipos de polimento

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Abstract
Polishing is an important procedure which takes part in the periodontal disease treatment, being commonly performed with the help of prophylactic paste and provides smoothing of the tooth surface, hindering the adhesion of new plaque. With this study we aimed to evaluate by means of scanning electron microscopy (SEM), the effects of dental polishing observing, qualitatively, the effectiveness and the damage, in three distinct treatments after calculus removal. Twenty (20) teeth were used (four of each dog), from which were obtained three samples each. The sixty samples were distributed into three groups (G0= tooth segmentation submitted to prophylaxis without polishing; G1= vestibular surface prophylaxis followed by polishing with the use of Defengy OC® and G2= vestibular surface prophylaxis followed by polishing with the use of pumice and fluorine gel). The samples were prepared and sent to images execution via SEM. These images, with enlargement of 100x and 500x, were evaluated and the classification averages were obtained. The statistical analysis of these averages was made through the Friedman nonparametric test using the software R. Statistical differences were observed (P<0,05) between groups 0 and 1 in the 100x magnification, whereas in the 500x magnification there was no difference (P>0,05) between the groups. The evaluation based on SEM images at two magnifications was essential, as it allowed the visualization of grooves and remaining calculi in a comprehensive way at 100x magnification and in more detail at 500x.
Keywords: tooth calculus; periodontal disease; veterinary dentistry; dental plaque; dental surface

Resumo
O polimento é um procedimento importante que faz parte do tratamento da doença periodontal, sendo comumente realizado com auxílio de pasta profilática e, propicia o alisamento da superfície dental, dificultando a adesão de nova placa bacteriana. Com esse estudo, objetivou-se avaliar por meio da microscopia eletrônica de varredura (MEV) os efeitos do polimento dental, avaliando qualitativamente, a eficácia e o dano, em três tratamentos distintos, após a remoção dos cálculos dentários. Foram utilizados 20 dentes (quatro de cada cão), de onde se obtiveram três amostras de cada. As 60 amostras foram distribuídas em três grupos (G0= segmentos dentários submetidos à profilaxia sem polimento; G1= profilaxia da face vestibular seguida de polimento com utilização de Defengy OC® e G2= profilaxia da face vestibular seguida de polimento com utilização de pedra pomes e flúor gel). As amostras foram preparadas e enviadas para realização das imagens por MEV. Estas imagens, com ampliação de 100x e de 500x, foram avaliadas e as médias de classificação obtidas. A análise estatística dessas médias foi feita por meio do teste não paramétrico de Friedman, utilizando o software R. Observou-se diferença estatística (P<0,05) entre os grupos 1 e 0 na magnificação de 100x, já na magnificação de 500x não houve diferença estatística (P>0,05) entre os grupos. O polimento foi eficaz ao tornar a superfície do esmalte dental lisa e regular reduzindo as ranhuras provocadas pela limpeza e retirou as granulações de cálculo remanescentes. A avaliação a partir das imagens de MEV em duas ampliações foi fundamental, por ter propiciado a visualização de ranhuras e cálculos remanescentes de forma abrangente na magnificação de 100x e mais detalhadamente na de 500x.
Palavras-chave: cálculo dentário; doença periodontal; odontologia veterinária; placa dentária; superfície dental

1. Introduction

Periodontal disease is one of the most commonly diagnosed diseases in small animals in clinical visits around the world, and by two years of age, it is estimated that 70% of cats and 80% of dogs already have some degree of periodontal disease. In this condition, there is inflammation of the periodontium caused by the presence of plaque, also called biofilm, which is observed as adherent material and yellowish color that forms on the surface of the tooth enamel and throughout the oral cavity[1,3]. The persistence of this plaque leads to the formation of dental calculus, due to mineralization

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resulting from the precipitation of calcium salts, composed of calcium carbonate and calcium phosphate, besides other minerals present in saliva[4,5]. Bacterial plaque formation is a result from colonization and growth of microorganisms on the dental surface, of which Porphyromonas gingivalis is one of the main etiologic agents frequently associated with periodontal disease. Studies demonstrate that the use of immunotherapy using Immunoglobulin Y (IgY-PG), obtained from chicken eggs, against the gingipain of Porphyromonas gingivalis were effective in preventing the attachment and proliferation of this bacterium, contributing to decrease plaque and calculus rates[6].

The removal of dental plaques and calculus must be done in two regions, supragingival and subgingival. In supragengival regions, manual instruments such as calculus extractors can be used or mechanical instruments such as an ultrasound machine; whereas in subgengival regions, the removal must be done with a curette. These procedures might cause damage to the tooth enamel, making it irregular, with presence of microbrasions or grooves, therefore it is necessary to polish this surface[7,8].

Polishing the surface of teeth that have been cleaned is done to remove any remaining plaque and to smooth out small grooves and dents, which may predispose to plaque deposition. Polishing is generally performed with a rubber cup and polishing paste. The pressure used ought to be gentle and at a speed of less than 3000 revolutions per minute (rpm), in addition to keeping these instruments in contact with this surface for a short period of time in order to avoid thermal or structural damage[7].

This study aimed to evaluate, by means of scanning electron microscopy (SEM), the effects of dental polishing in dogs, in order to rate qualitatively, efficacy and damage, after the removal of dental calculus, in three different treatments

2. Material and methods

This study was conducted in the Veterinary Dentistry Laboratory of the Veterinary Hospital of the School of Veterinary and Animal Sciences of the Federal University of Goiás (UFG) and approved by the Ethics Committee on Animal Use under protocol number 031/17. For the research, teeth from five dogs with similar degree of periodontal disease that have died, from the Zoonosis Control Center of Goiânia, were used. From each animal four teeth were used, the bilateral mandibular first molar and the bilateral maxillary fourth premolar, which had dental calculus on the enamel surface of the tooth. Three groups were formed with 20 samples each. The Control Group (G0) was composed of segments submitted to calculus removal without polishing; Group 1 (G1), of segments subjected to calculus removal and polishing with Defengy OC a mineral vitamin supplement composed of powdered egg, essential oils of rosemary and clove and other elements in the form of an edible paste, and Group 2 (G2) by the segments submitted to removal of calculus and polishing with Quimidrol® pumice stone and Flugel® fluoride gel totalizing 60 samples (three sections from each of the 20 teeth).

These teeth were also submitted to cleaning and removal of dental calculus using a Microdont® high-speed ultrasonic scraper. Afterwards, polishing was performed with a Microdont® fine-grained rubber cup for ten seconds on each tooth segment, based on the time used by Fichtel(5), except for the lingual/palatal surfaces, which represent the G0. The meso-buccal enamel was polished with Defengy OC® (G1), while the distal-buccal enamel was polished with Quimidrol® pumice and Flugel® fluoride gel (G2). The cleaning and polishing procedures were performed by a single experienced professional to avoid variations in technique. After polishing, the tooth fragments were odontosectioned in the transition region between root and crown, with a medium grain flame diamond bur KG Sorensen®, for dental cuts in 3 x 3 mm segments each. An odontosection of the palatal or lingual surface was also performed for G0 sampling. Therefore, the evaluation of different polishing materials in samples of the same dental origin was guaranteed.

Subsequently, the fragments were dehydrated in progressive alcohol solutions, starting in 70% alcohol solution, transferring to 80%, 90%, 95%, and 100% alcohol, remaining in each concentration for 30 minutes. After dehydrated in 100% alcohol, the segments were stored, wrapped in kraft paper, in vials with blue silica gel. The specimens were taken to the High Resolution Microscopy Multipurpose Laboratory (LabMic) of the Federal University of Goiás, where they were fixed on the metal base (stub) with double-sided carbon tape (conductive tape). The electrical contact from the top of the sample to the metallic base was made with a carbon ink wire. After drying, the sample was coated with gold using a film deposition system, Denton Vacuum, Desk V, and analyzed with a scanning electron microscope (SEM), Jeol, JSM - 6610, equipped with EDS, *Thermo Scientific NSS Spectral Imaging*.

LabMic obtained 120 images, of which 60 had 100x magnification and 60 with 500x magnification. The images were randomly distributed and sent to seven examiners, who were unaware of the process and purpose of the work, for evaluation as to efficacy and damage. Criteria for image evaluation were established, based on the one described by Fichtel[5],and described in Chart 1. The examiners received the images obtained by SEM and two images representing scores between one and five for both efficacy and damage.

Chart 1. Description of the scores related to the criteria used (efficacy and damage) for the evaluation of the images of the tooth surface obtained by scanning electron microscopy, in teeth submitted to different polishing

Scores	Efficiency	Damage
1	Absence of granules on the enamel surface	Absence of grooves in a microscopic image field
2	Up to 10% granules on the enamel surface	Up to 10 grooves in number. present in a microscopic image field
3	10 to 25% granules on the enamel surface	Up to 30 grooves seen in a microscopic image field
4	25 to 40% granules on the enamel surface	Grooves abundantly seen in a microscopic imaging field
5	More than 40% granules on the enamel surface	Grooves under the entire tooth enamel surface

Source: Adapted from Fichtel (2008, p. 234).

Thus, each image was rated seven times for efficiency and seven times for damage. In other words, two values were assigned to each image by each of the seven examiners, which were organized in four tables: two tables for efficiency and damage at 100x magnification, and another two tables for efficiency and damage at 500x magnification. From these data, the average classification of each image was conducted. The statistical analysis was performed considering the variables efficacy and damage, with 100x and 500x magnification, referring to the groups G0, G1 and G2, using Friedman's non-parametric test.

3. Results and discussion

Sixty samples were evaluated, obtained from 20 teeth of five dogs. The groups were divided into G0, G1 and G2, each with 20 samples, totalizing 60 samples, which were observed at 100x and 500x magnification, totaling 120 images. After the evaluation by the seven examiners, 840 values were obtained for efficiency and 840 values for damage. There was variation in the evaluation assigned by the examiners. The following are images obtained in this study by SEM at 100x and 500x magnification, representative of the three groups studied (Figures 1 and 2).

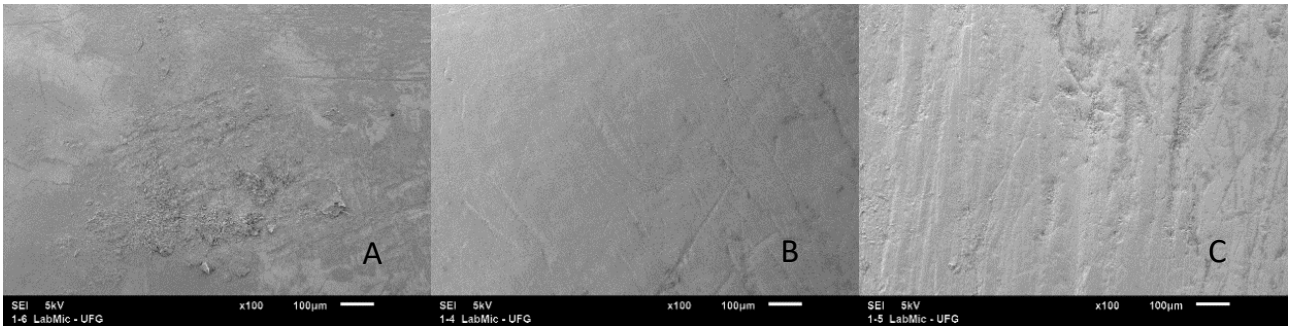

Figure 1. Scanning electron microscopy (SEM) images of canine tooth enamel submitted to periodontal treatment with and without polishing. (A) Group without polishing G0; (B) polishing with Defengy OC® paste G1; (C) polishing with Quimidrol® pumice stone and Flugel® fluoride gel G2. Magnification: 100x. Scale: 100µm.

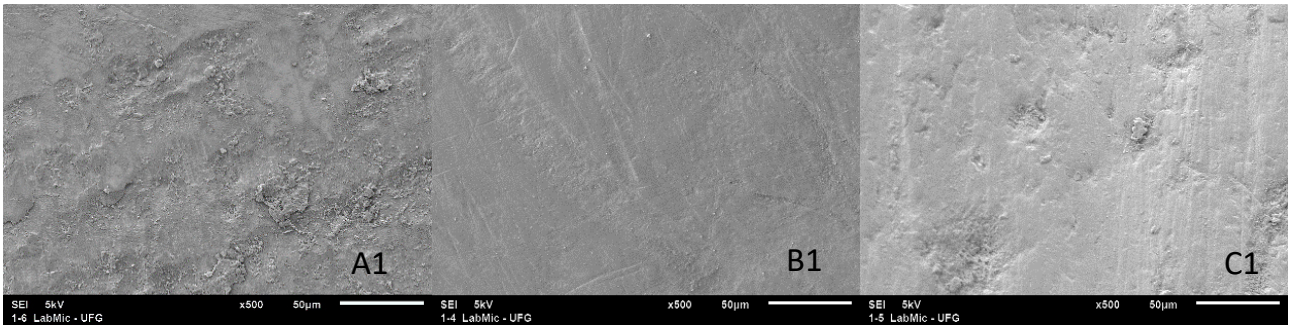

Figure 2. Scanning electron microscopy (SEM) images of canine tooth enamel submitted to periodontal treatment with and without polishing. (A1) Group without polishing G0; (B1) polishing with Defengy OC® paste G1; (C1) polishing with Quimidrol® pumice stone and Flugel® fluoride gel G2. Magnification: 500x. Scale: 50µm.

When evaluating the images obtained by SEM, it could be observed that in G0, the removal of dental calculus caused the formation of grooves on the dental enamel surface (images A and A1 of figures 1 and 2, respectively), it was also observed that visually there is considerable amount of remaining calculus. In G1, in which dental samples polished with oral paste are represented, in both images B and B1 of figures 1 and 2 respectively, sparse granular remnants of calculus and the presence of distinguishable grooves on the surface, interspersed with smooth areas, are observed. In G2, the samples polished with pumice stone and fluoride gel, when comparing images C and C1 of figures 1 and 2 respectively, it was possible to notice surfaces with a smaller amount of dental calculus in relation to G0, but with clear grooves in the microscopic image field. To better evaluate the means and thus cover all the samples, a statistical analysis was performed using the Friedman non-parametric method, by means of the R software. The score values obtained are expressed as median (Chart 2).

Chart 2. Results of the statistical analysis using Friedman's nonparametric method.

Variable	Groups			P-value
	G0	G1	G2	
Efficiency (100x)	3.49[a]	2.42[b]	2.57[ab]	0.0048
Damage (100x)	3.40[a]	2.45[b]	2.85[ab]	0.0437
Efficiency (500x)	3.21[a]	2.71[a]	2.7[a]	0.0729
Damage (500x)	3.00[a]	2.45[a]	2.75[a]	0.2120

Values expressed as median for groups 0 (G0), 1 (G1) and 2 (G2). Significance level (P-value) used was 5%. (a/a, b/b= no statistical difference; a/b= statistical difference).

As for the efficiency variable, evaluated at 100x magnification, there was no statistical difference ($p>0.05$) between G0 and G2, that is, between the group without polishing and the group submitted to polishing with pumice stone and fluoride gel. Similarly, G1 treated with oral paste showed no statistical difference ($p>0.05$) with G2. However, regarding G0, G1 showed statistical difference ($p<0.05$).

When observing the damage variable, at 100x magnification, the same occurrence was noted, there is no statistical difference ($p>0.05$) between G0 and G2, and between G1 and G2. However, there is a statistical difference ($p<0.05$) between G0 and G1,that is, between the group not subjected to polishing and the group in which polishing was performed with oral paste. Therefore, considering the images at 100x magnification, when comparing the three groups among themselves, there was a statistical difference ($p<0.05$) for the variables efficiency and damage of G1 in relation to G0, which had lower efficacy and greater damage. G1 had the best evaluation for efficacy and the worst for damage. G2 showed no statistical difference with G0 and G1.

At 500x magnification there was no statistical difference ($p>0.05$) between the groups, seeing that when the image is magnified the area in the microscopic field is smaller and more restricted, although it is presented with more detail, which may explain the statistical difference only between the groups observed at 100x magnification. Thus, the use of two different magnifications was important, because in images with lower magnification it was possible to observe more comprehensively and note the better result of G1, when compared to G0, and in the images at higher magnification it was possible to observe the samples in a more detailed and approximate manner, which allowed the remnants kept at the end of the procedure to be seen.

Both treatments applied, polishing with oral paste and polishing with pumice stone and fluoride gel, proved to be effective in removing residual granules. This was evidenced in the qualitative analysis of the SEM images, in which groups G1 and G2 showed enamel surfaces with few granules of small diameter in contrast to the same analysis done in G0, which, due to the fact that it had not been subjected to polishing, showed abundant granules, often over the entire microscopic image field.

Toriggia et al.[9] as well as Pameijer et al.[10] asserted that larger magnifications of SEM images improve the interpretation of results, which is in agreement with what was observed in this study in which dental grooves and granular residues were more clearly visualized when the image was magnified, refining the analysis of treatment effectiveness. However, the 500x magnification images cover a restricted area of the enamel surface, and in order to analyze the enamel as a whole, the 100x magnification allowed a better comparison of the treatment on a larger area of the tooth surface, and therefore, more representative of the tooth as a whole.

In their study, Pameijer et al. [10] evaluated SEM images of 25 dental surfaces characteristics. Among them, 10 were manually treated with Hu Friedy Gracey curette, other 10, with Cavitron ultrasonic unit with P 10 tip, and the remaining five formed the control group. The results obtained by these researchers were similar for both methods when analyzing images at higher magnifications, similarly to what was observed in this study, in which the images at higher magnifications showed no statistical difference in the results.

Toriggia et al.[9] in their comparative study of four treatments, concluded that the removal of dental calculus by ultrasonic cavitator associated with polishing was the most efficient method of dental cleaning, with no difference between the other groups, in which cleaning was performed with ultrasonic cavitator without

polishing, only curettage and curettage associated with polishing. Thus, it has already been reported the effectiveness of the joint use of dental cleaning by the use of ultrasonic cavitator and polishing, in accordance with what applied in this study.

The use of SEM to study the tooth surface allowed the observation of the state of involvement of the teeth of these animals by microorganisms, besides allowing the visualization of the effectiveness of the respective treatments. Cobb et al. also used SEM to evaluate residues of dental calculus and biofilm in the subgingival region after the use of EDTA gel, noting the change in structure of the biofilm on the tooth surface after treatment[11]. The oral paste used in this study was developed with the purpose of being administered as a vitamin mineral supplement for dogs and cats, being given after meals, in order to reduce plaque formation and halitosis; its formulation contains egg powder and has already been used in another study with periodontal disease, due to the action of IgY- PG, whose results showed a reduction in plaque and dental calculus indices and no halitosis[12]. It was proposed, through this study, a different form for its application due to the presence of microcrystals in its composition, which also showed effective results in cleaning the dental surface with decreased presence of dental calculus.

4. Conclusion

Dental polishing was effective in making the tooth enamel surface smooth and regular, reducing the grooves caused by periodontal treatment and removed the granulations of remaining calculus. Polishing methods using commercial oral paste and pumice stone with fluoride gel proved efficient in making the dental enamel surface smooth, enabling less adhesion of dental plaque and calculus, with the better performance of the oral paste, which provides smoother surface and less damage to the enamel. The evaluation using SEM images was fundamental, as it allowed the visualization of grooves that could not be seen with the naked eye, which enabled the analysis of the damage caused by cleaning, and of the tooth surface after polishing, making it possible to compare the efficiency of different treatments, and the real need to polish the teeth. The two different magnifications allowed for a more accurate examination, as a result of visualization in greater amplitude, and subsequently in a more approximate and detailed scale.

Conflict of interests

Authors declare no conflicts of interest.

Author contributions

Conceptualization: N. M. S. Correia and P. L. S. N. Guimarães. *Data curation*: N. M. S. Correia and V. E. A. Barros. *Formal Analysis*: E. Arnhold. *Investigation*: V. E. A. Barros. *Project management*: P. L. S. N. Guimarães. *Supervision*: M. C. S. Fioravanti. *Writing (original draft)*: N. M. S. Correia. *Writing (revision and edition)*: R. C. Carneiro.

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