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ORIGINAL RESEARCH

Biocorrosion of Endodontic Files through the Action of Two Species of Sulfate-reducing Bacteria: *Desulfovibrio desulfuricans* and *Desulfovibrio fairfieldensis*

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ABSTRACT

Aim: This study assessed the biocorrosive capacity of two bacteria: *Desulfovibrio desulfuricans* and *Desulfovibrio fairfieldensis* on endodontic files, as a preliminary step in the development of a biopharmaceutical, to facilitate the removal of endodontic file fragments from root canals.

Materials and methods: In the first stage, the corrosive potential of the artificial saliva medium (ASM), modified Postgate E medium (MPEM), 2.5 % sodium hypochlorite (NaOCl) solution and white medium (WM), without the inoculation of bacteria was assessed by immersion assays. In the second stage, test samples were inoculated with the two species of sulphur-reducing bacteria (SRB) on ASM and modified artificial saliva medium (MASM). In the third stage, test samples were inoculated with the same species on MPEM, ASM and MASM. All test samples were viewed under an infinite focus Alicona microscope.

Results: No test sample became corroded when immersed only in media, without bacteria. With the exception of one test sample between those inoculated with bacteria in ASM and MASM, there was no evidence of corrosion. Fifty percent of the test samples demonstrated a greater intensity of biocorrosion when compared with the initial assays.

Conclusion: *Desulfovibrio desulfuricans* and *D. fairfieldensis* are capable of promoting biocorrosion of the steel constituent of endodontic files.

Clinical significance: This study describes the initial development of a biopharmaceutical to facilitate the removal of endodontic file fragments from root canals, which can be successfully implicated in endodontic therapy in order to avoiding parendodontic surgery or even tooth loss in such events.

Keywords: Biocorrosion, Dental instruments, Desulfovibrio, Endodontic files, Microbiology, Sulfate-reducing bacteria.

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INTRODUCTION

The complex anatomy of the root canal system, varying in shape and size, limits the success of endodontic treatment. Besides this morphological complexity, during root canal treatment the space previously occupied by the pulp must be filled and then instrumented, so that voids are eliminated completely before the apical foramen is sealed. Furthermore, endodontic treatment becomes even more complex and uncertain if an endodontic file fracture occurs.[1,2] The unsuccessful removal of such a fragment can lead to tooth loss as well as the need for endodontic retreatment in the presence of signs and symptoms, indicating the continuity of infection.[3-5]

In order to remove this metal fragment, various techniques and maneuvers are employed during treatment, with successful fragment removal in most cases.[6-8] However, no technique is completely safe since, perforations, destruction or reduction in the resistance of the tooth root can occur.[9,10]

Manual endodontic files are instruments manufactured with austenitic stainless steel alloys and used in root canal treatment to eliminate organic substrates, debris and microorganisms.[2,11] These instruments are relatively resistant to corrosion as they have chromium in their microstructure. The chromium added to stainless steel, in contact with air or oxygenated solutions, forms a film of chromium oxide adhering to the surface making the instrument waterproof, with greater hardness and density, and possessing a capacity of repair. However, these characteristics can be neutralized in reducing environments, such as immersion of the instrument in chlorinated solutions, which occurs frequently during endodontic treatment.[2,12-14] After the disruption of the passivating film of chromium oxide, corrosion begins. This brings about destruction of the cutting edges resulting in the loss of cutting efficiency, therefore, increasing the risk of fracture of the instrument within the root canal.[2]

Given this, it is possible to consider the repeated corrosion of an endodontic file fragment within the root canal as a process that facilities the removal of the fragment. Apart from this type of corrosion (inorganic), there is biocorrosion which occurs by the corrosive action of microorganisms, such as sulfate-reducing bacteria (SRB), which participate actively in the corrosive process by initiating or accelerating the electrochemical reaction of metal dissolution.[15]

The SRB are strictly anaerobic, with an optimum temperature range between 25 and 44°C and a pH between 5.5 and 9.0. Currently, there are over 20 well-known genera, such as *Desulfovibrio, Desulfomonas, Desulfotomaculum, Desulfolobus, Desulfobacter, Desulfococus, Desulfosarcina, Desulfonema*, etc.[15-20] These fastidious microorganisms are difficult to isolate and identify.[21,22] They can be found in the environment, soil, freshwater and salty marshes or in the human body, mainly in the intestinal flora, where the species *Desulfovibrio desulfuricans* can be frequently detected.

The objective of this study was to assess the biocorrosive capacity of two species of SRB (*Desulfovibrio desulfuricans and Desulfovibrio fairfieldensis*) on endodontic files as a preliminary step in the development of a biopharmaceutical (initially referred to as BACCOR-F and BACCOR-D) to facilitate the removal of endodontic file fragments from root canals.

MATERIALS AND METHODS

The project was submitted to the Ethical Research Committee of the Faculty of Medicine and the Hospital, Universitário Antônio Pedro of Fluminense Federal University and approved by document CEP CMM/HUAP nº 185/09.

Sample Selection

Two SRB isolates from human saliva; the species *D. desulfuricans* and *D. fairfieldensis* (in corsortium as it was not possible to isolate the latter strain due to loss of cellular activity after isolation) and a sample of the species *D. desulfuricans*, isolated from the environment were chosen from the stock strains at the laboratory of biocorrosion and biodegradation (LABIO) of the National Institute of Technology (INT).

Chemical Analysis of Kerr-type Files

Two samples of Kerr-type nº 80 endodontic files, 31 mm (Dentsply Ind. and Com. Ltda.; Maillefer Instruments, Swiss, LOTE: 1688420, REF: A 012B 031 080 00) were sent to the lab (Labmat® group, analysis and testing) for chemical analysis. Chemical composition analysis by combustion and quantitative analysis by X-ray fluorescence spectrometry were carried out in order to identify the American Iron and Steel Institute (AISI) classification of the steel of the file.

Assembly of Test Samples

Ten Kerr nº 30 files (31 mm; LOT: 390785, REF: A 012B 0031 0030; LOT: 3776580, REF: A 012D 031 030 00 k LOT: 3776580, REF: A 012D 031 030 00) and two Kerr n° 80 files (31 mm; LOT: 1688420, REF: A 012B 031 080 00) were chosen as test samples. The endodontic files were embedded in acrylic resin for subsequent cross-sectioning by a microcut machine (Mecatome T180, Presi, France). Each test sample was sanded and polished. All test samples were observed under an infinite focus microscope (Alicona imaging, Grambach/Graz, Austria) and the obtained images were analyzed, processed and stored by a digital image processing program (Infinite focus opitical 3D surface metrology). The test samples were sterilized for 20 minutes at 121°C and then used to evaluate the corrosive potential of the culture media and the species *D. desulfuricans* and *D. fairfieldensis* (Fig. 1).

Biocorrosion Tests

To carry out the biocorrosion or immersion tests of the samples, the following media were prepared:
Modified postgate E culture medium (MPECM): Indicated for the growth and isolation of SRB, composed of the

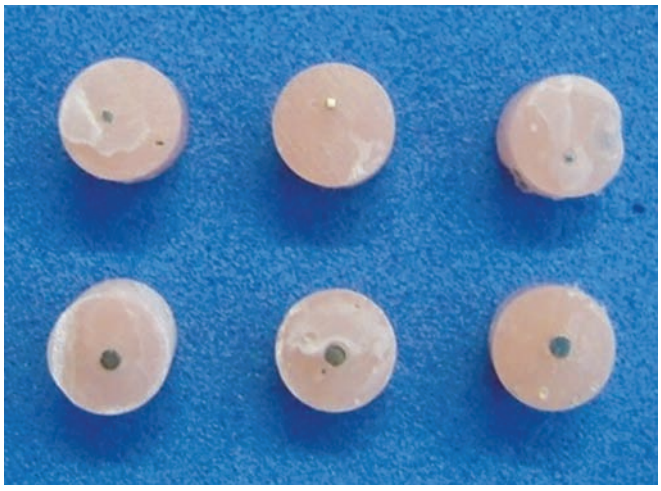


Fig. 1: Test samples

following (gm/liter of distilled water)—KH_2PO_4 (0.5), NH_4Cl (1.0), Na_2SO_4 (1.0), $CaCl_2{\cdot}_2H_2O$ (0.67), $MgCl_2{\cdot}_6H_2O$ (1.68), sodium lactate (7.0), yeast extract (1.0), ascorbic acid (0.1), agar-agar (1.9), NaCl (5.0), rezasurina (4.0 ml) and $FeSO_4{\cdot}7H_2O$ (0.5), with constant stirring and nitrogen purging.[17] The pH of the medium was adjusted to 7.6 with NaOH. Once prepared, 10 ml of medium was distributed in penicillin-type flasks with a 15 ml volume capacity, purged with N_2 for 20 seconds, sealed with a rubber stopper and aluminum, and then sterilized for 15 minutes at 121°C (Prismatec-autoclaves, Autoclave vertical/cs). All test samples were stored in a fridge (Electrolux-frostfree DF45-premium) at 4°C until the moment of the test.

Artificial saliva medium (ASM): Composed of (gm/l of distilled water) – 0.1256 NaCl, 0.9639 KCl, 0.1892 KSCN, 0.6545 KH_2PO_4, 0.2 Urea, 0.5832 Na_2SO_4, 0.178 NH_4Cl, 0.2278 $CaCl_2{\cdot}2H_2O$ and 0.6308 $NaHCO_3$.[23] The pH of the medium was adjusted to 6.8 with NaOH. A modified artificial saliva medium (MASM) was also prepared following the same methodology and composition as the artificial saliva medium, with a substitution of an Na_2SO_4 quantity of 0.5832 gm/l to 1.0 gm/l in order to stimulate the growth of SRB by means of a greater amount of sulfate to perform a reduction to sodium sulfide. Once prepared, these media were distributed and stored in the same way as reported above.

In addition to the culture media, a solution of sodium hypochlorite 2.5% (NaOCl) (Biodynamic chemistry and pharmaceuticals LTDA) and white medium (a control of media without solution) were prepared for the immersion test.

Evaluation of the Corrosive Potential of the Culture Media

In the first step, the corrosive potential of culture media (ASM, MASM and MPECM), white medium and sodium hypochlorite (NaOCl) 2.5% solution on the Kerr n°30 endodontic file samples was assessed by immersion tests.

The test consisted of the immersion of four different groups of test samples in various culture media: nine test samples in group G1 (MPECM), nine test samples in group G2 (ASM), three test samples in group G3 (2.5% sodium hypochlorite solution) and three test samples in group G4 (white medium). These media were purged with N_2 for 10 seconds and incubated for 28 days at 30°C. After the incubation period, the test samples were viewed under an infinite focus microscope, following the same methodology used for the previous images. The 'before' and 'after' test images were compared to examine the occurrence of areas and pits of corrosion on the metal surfaces of the test samples.

Evaluation of the Corrosive Potential of the Species *D. desulfuricans* and *D. fairfieldensis*

In the second step, the biocorrosive capacity of the species *D. desulfuricans* and *D. fairfieldensis* (in consortium), inoculated in ASM and MASM together with the immersed Kerr n° 80 files, was evaluated.

After following the same methodology for the inoculation of the culture media as reported above, the inoculation of each bacterial strain was performed by transferring a 1 ml aliquot of the original culture (MPECM with the cultivated bacteria) to a reducing solution medium for anaerobic bacteria. Then, a 1 ml aliquot was transferred to each test group. For the G7 group, the inoculation of *D. fairfieldensis* (in consortium) was performed directly, by transferring a 1 ml aliquot of bacterial culture to the modified artificial saliva medium. All samples were incubated at 30°C for 28 days in an incubator.

In the third step, Kerr n°30 files were chosen. Six test samples each were included in groups G10 (MPECM inoculated with *D. fairfieldensis* in consortium) and G11 (MPECM inoculated with *D. desulfuricans*), and three test samples each in groups 'G12' (ASM inoculated with *D. fairfieldensis* in consortium), G13 (ASM inoculated with *D. desulfuricans*), G14 (MASM inoculated with *D. fairfieldensis* in consortium) and G15 (MASM inoculated with *D. desulfuricans*).

Following the same methodology as above, a 1 ml aliquot of each bacterial strain was inoculated directly from their original cultures (in MPECM) to groups G10 and G11. For groups G12, G13, G14 and G15, bacterial cells from the original cultures (in MPECM) were washed and the resulting precipitates were inoculated into each respective group. All inoculated test samples were incubated for 28 days at 30°C in an incubator.

After the incubation period, the test samples for the second and third steps were removed from the culture media. Chemical stripping was performed to remove

the products of corrosion and impurities present on the metal surface. The test samples were viewed under an infinite focus microscope, using the same methodology as mentioned previously for comparison of the 'before' and 'after' images in and between the groups.

A bacterial activity test was carried out for groups G5, G6, G7, G8, G9, G12, G13, G14 and G15, after 15 days to evaluate the ASM and MASM. A 1 ml aliquot from each group was removed and inoculated in 10 ml of MPECM in a penicillin-type flask. They were incubated for 28 days at 30°C in an incubator. After this time period, the bacterial activity of each spike from the respective groups was evaluated using the biocorrosion assays.

RESULTS

Classification of the Steel Type of the Kerr Endodontic Files

By chemical analysis of two samples of Kerr nº 80 type endodontic files, it was possible to classify the steel as AISI 304, based on the elements in the steel, which are shown in Tables 1 and 2. It was verified that one of the characteristics of this type of steel is its inability to resist corrosion.[18,24]

Table 1: Chemical composition by combustion

Elements (weight %)	*C*	*S*
Obtained	0.079	0.001

C: Carbon; S: Sulfur

Table 2: Quantitative analysis by X-ray fluorescence spectrometry

Elements (weight %)	*S*	*Mn*	*P*	*Cr*
Obtained	1	1.63	0.017	18.12
Elements (weight %)	N	Mo	Cu	Fe
obtained	8	0.65	0.18	Base

Si: Silicon; Mn: Manganese; P: Phosphorus; Cr: Chromium; Ni: Níckel; Mo: Molybdenum; Cu: Copper; Fe: Iron

Biocorrosion Test

Evaluation of the Corrosive Potential of the Culture Media

A comparison of the images obtained before and after the biocorrosive test revealed that there was no corrosion of the test samples from groups G1 to G4 (Table 3). Figure 2 shows a 'before' and 'after' image of a test sample in MPECM with the absence of corrosion on the metal face of the cross-section. The test samples immersed in 2.5% sodium hypochlorite (G3) also showed no evidence of corrosion.

Evaluation of the corrosive potential of the Species D. desulfuricans and D. fairfieldensis

Table 4 shows the results for groups G5 to G9. The first images from the immersion test of the groups revealed that in G5 there was formation of a small pit of corrosion along the edges of the test sample, with other areas of the cross-section surface remaining unchanged; in G6, there was formation of four small pits of corrosion on the metal surface; in G8, one pit of corrosion was observed (large in size as compared to the others) due to the previous presence of a small defect on the test sample, along with another smaller pit of corrosion, also originating from the previous defect. In G9, five small pits developed on the metal surface and in G7 there was no sign of corrosion, leaving the final image unchanged as compared to the initial.

The results from groups G10 to G15 are presented in Table 5. The resulting images from the final biocorrosion test (groups G10 to G15) suggest a greater degree of corrosion as compared to the previous test (G5 to G9). Groups G10 and G11 showed a greater intensity of corrosion as compared with the other groups, followed by groups G12

Table 3: Distribution of biocorrosive test groups for the evaluation of corrosive potential of culture media

Groups	*Medium*	*Inoculated bacteria*	*Number of test samples used*	*Metal surfaces observed*	*Number/frequency (%) of surfaces with corrosion*
G1	Modified postgate E medium	No	9	9	0
G2	Artificial saliva	No	9	9	0
G3	Sodium hypochlorite	No	3	3	0
G4	White médium	No	3	3	0

Table 4: Distribution of the culture media and inoculated bacteria in each group to evaluate the biocorrosive potential of the species *D. desulfuricans* and *D. fairfieldensis*

Groups	*Medium*	*Inoculated*	*Number of test samples used*	*Metal surfaces observed*	*Corrosion of surface*
G5	Artificial saliva	*Desulfovibrio desulfuricans*	1	1	Yes
G6	Artificial saliva	*Desulfovibrio fairfieldensis* in consortium	1	1	Yes
G7	Modified artificial saliva	*Desulfovibrio fairfieldensis* in consortium	1	1	No
G8	Modified artificial saliva	*Desulfovibrio fairfieldensis* in consortium	1	1	Yes
G9	Modified artificial saliva	*Desulfovibrio desulfuricans*	1	1	Yes

Table 5: Distribution of culture media and inoculated bacteria in each group (after washing the bacteria) for the evaluation of the biocorrosive potential of the species *D. desulfuricans* and *D. fairfieldensis*

Groups	*Medium*	*Inoculated bacteria*	*Number of test samples used*	*Metal surfaces observed*	*Number/frequency (%) of surfaces with corrosion*
G10	Modified postgate E	*Desulfovibrio fairfieldensis* in consortium	6	12	11 (91.7)
G11	Modified postgate E	*Desulfovibrio desulfuricans*	6	12	11 (91.7)
G12	Artificial saliva	*Desulfovibrio fairfieldensis* in consortium	3	6	4 (66.7)
G13	Artificial saliva	*Desulfovibrio desulfuricans*	3	6	3 (50)
G14	Modified artificial saliva	*Desulfovibrio fairfieldensis* in consortium	3	6	4 (66.7)
G15	Modified artificial saliva	*Desulfovibrio desulfuricans*	3	6	3 (50)

and G14. Groups G13 and G15 had a lesser intensity of corrosion shown by a greater number of images without alterations (three images for each group), and the images show fewer and smaller pits of corrosion as compared to the other groups. Groups G10 and G11 had only one image for each group without surface alterations, while groups G12 and G14 had two.

The images suggesting corrosion had: (1) isolated or multiple small pits of various sizes and shapes, localized on the borders or on the cross-sectioned metal surface of the test sample (Fig. 3), and (2) extensive areas, on the border of the test samples, of visible depressions or irregular corrosion in some images, extending toward the center of the test samples and/or along the whole border (Fig. 4).

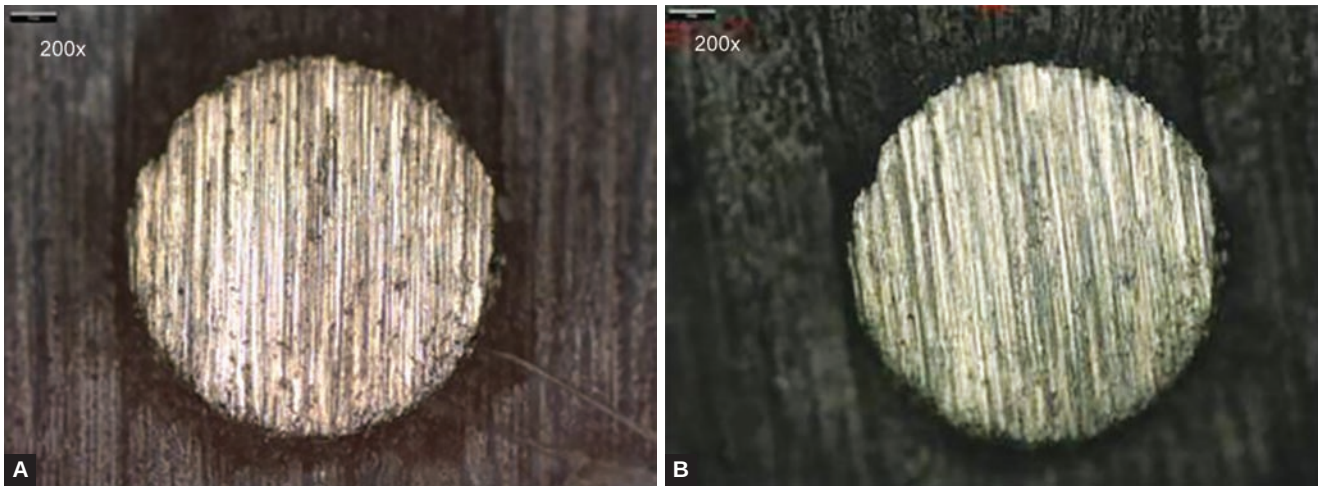
The images suggesting corrosion along the borders of the test samples were more frequent and extensive when compared to other types of corrosive defects (Fig. 5). Furthermore, in areas where the 'before' images indicated structural defects, there was the appearance of extensive areas of corrosion. The previous defects localized at the borders of the test samples were those that had the greatest corrosion, with the formation of extensive corroded areas along the borders and/or extending toward the center of the test sample (Fig. 6). Other previous defects on the surface of the test samples also had corrosion after the assay, however, with lesser intensity as compared to areas of previous defects on the borders.

Evaluation of Artificial Saliva and Modified Artificial Saliva Media with D. Desulfuricans and D. Fairfieldensis

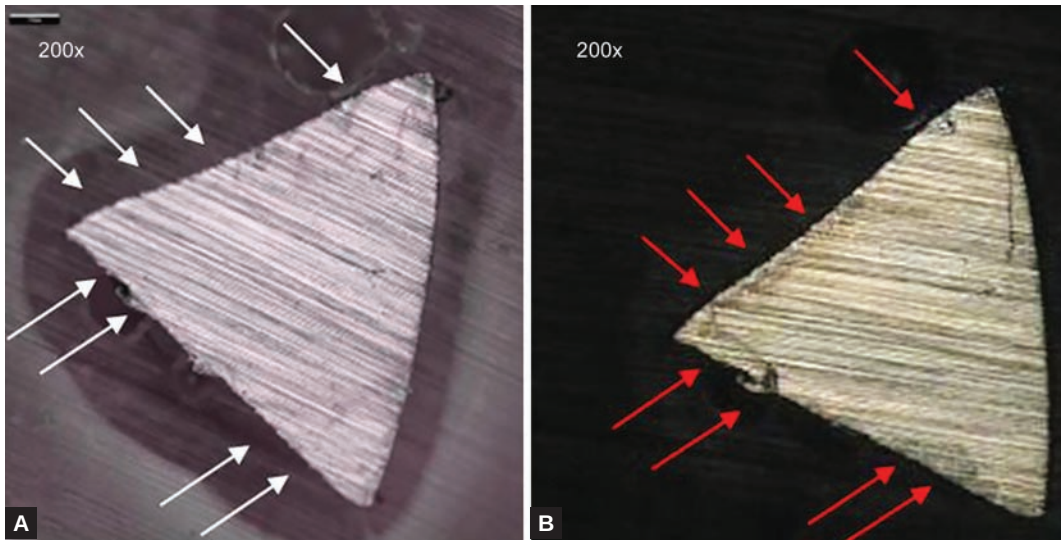
The ASM and MASM were unable to maintain activity of the *D. desulfuricans* and *D. fairfieldensis* species. This was proven on the 15th day when each test sample culture from groups G5, G6, G7, G8, G9, G12, G13, G14 and G15 was subcultured into MPECM. After 28 days of subculturing, there was no indication of bacterial activity in any subcultured groups. However, during the short period in which these strains maintained activity, there was formation of pits of corrosion in the test samples used in each group.

DISCUSSION

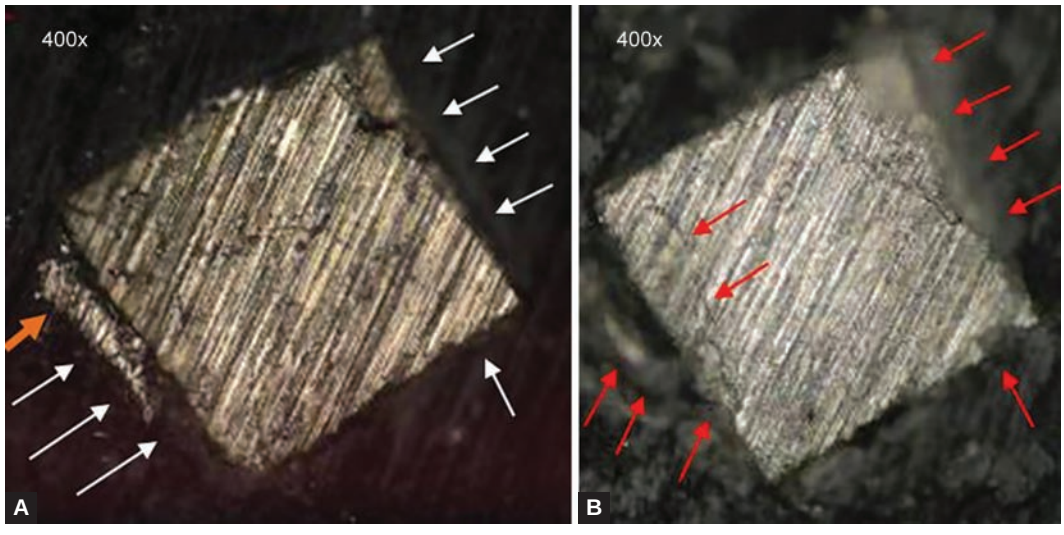
Many studies have demonstrated the presence of SRB in the oral and gastrointestinal microbiota, beyond their biocorrosive capacity on metal structures.[25-29] However, until now, no study has estimated the biocorrosive capacity of SRB on endodontic files. The present study estimates the



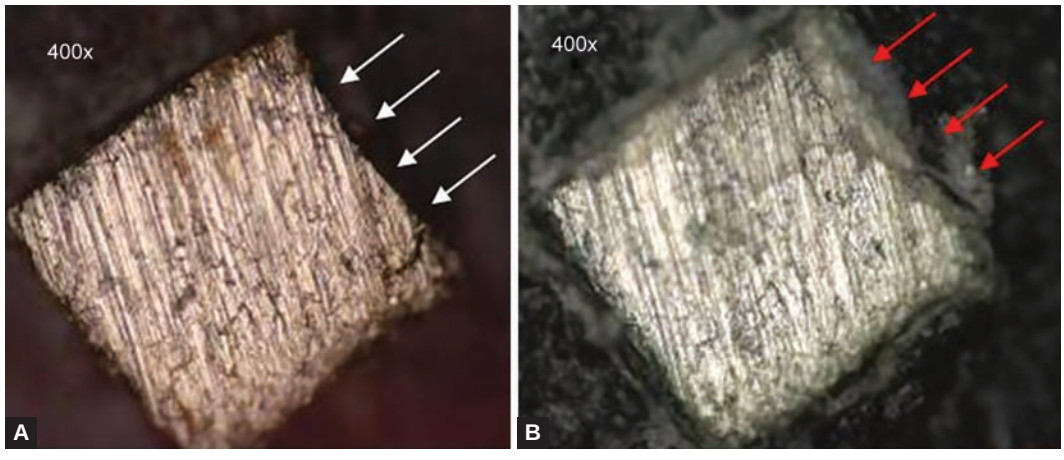
Figs 2A and B: A test sample from group G1 before (A) and after (B) immersion in modified Postgate E medium



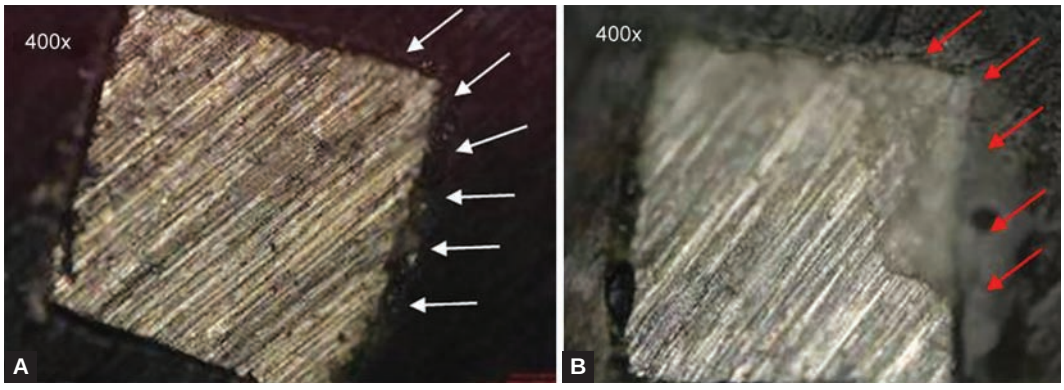
Figs 3A and B: A test sample from group G9 before (A) and after (B) the biocorrosion test in modified artificial saliva inoculated with *D. desulfuricans*. Indication of pits and areas suggestive of corrosion along the borders (red arrows) (B). White arrows indicate the same areas before biocorrosion (A)



Figs 4A and B: A test sample from group G10 before (A) and after (B) the biocorrosion test in modified postgate E medium, inoculated with *D. fairfieldensis*. Indication of pits and areas of corrosion along the edges (red arrows) and a metal fragment that disappeared after biocorrosion (B). The white arrows indicate the same areas and the orange arrow indicates the metal fragment before biocorrosion (A)



Figs 5A and B: A test sample from group G11 before (A) and after (B) the biocorrosion test in modified postgate E medium, inoculated with *D. desulfuricans*. Indication of pits and areas suggesting corrosion along the edges (red arrows) (B). The white arrows indicate the same areas before biocorrosion (A)



Figs 6A and B: A test sample from group G11 before (A) and after (B) the biocorrosion test in modified postgate E medium, inoculated *D. desulfuricans*. Indications of large areas suggesting corrosion along the edges and extending toward the center of the test sample (red arrows) (B). The white arrows indicate the same areas before biocorrosion

biocorrosive potential of SRB with the aim of developing a biopharmaceutical (initially referred to as BACCOR-F and BACCOR-D) to facilitate the removal of endodontic file fragments from the root canal.

Can a fractured endodontic file fragment within the root canal be removed by the biocorrosive capacity of SRB? This study cannot conclude as such; however, it is evident that to study the corrosive capacity of this group of bacteria on endodontic files is an important first step in resolving this question.

Identification of the steel type of the endodontic files is important since the chemical composition of the steel can directly influence the corrosive process, influencing the early stages of biofilm formation and the rate of accumulation and distribution of bacterial cells on the metal surface.[28,30] However, similarly to that observed in the current study, it appears that AISI 304 steel is not resistant to biocorrosion and areas of corrosion can form through the action of SRB.[31]

In the first immersion test, evaluation of the corrosive potential of the culture media showed that only group G3 had changes on the metal surface, while groups G1 (MPECM), G2 (ASM) and G4 (white medium without immersion) showed no points of corrosion or surface changes. With this data, it was possible to conclude that MPECM and ASM are not corrosive toward the metal components of the test samples, equaling the test samples from group G4 (white medium). The test samples immersed in 2.5% sodium hypochlorite solution (G3) also did not show points of corrosion; however, it was possible to observe changes in the coloration of the metal surface in two out of three test samples, where extensive areas of yellow coloration suggested the initial stage of oxidation of the metal surfaces. Videla reported the corrosion of AISI 304 steel in the presence of chloride, while other studies reported intense corrosion by sodium hypochlorite solutions at different concentrations (1.0, 4.0 or 5.25%), through immersion and electrochemical tests examined by scanning electronic microscope.[14,15,32] Parashos et al described corrosion in files immersed in sodium hypochorite under 30 minutes of ultrasound vibration, as irregular erosive cavities of variable dimensions, resembling honeycombs.[32] However, such assays were performed in an aerobic environment which favors steel corrosion when immersed in sodium hypochlorite solution, differing from what we observed in group G3, where the environment was anaerobic.

Next, we evaluated the corrosion caused by the species *D. desulfuricans* and *D. fairfieldensis* in consortium, in kerr-type n°80 endodontic files (G5, G6, G7, G8 and G9), in immersion assays using ASM and MASM. This objective of this step was to identify an alternative culture media which closely resembles the oral environment (biocompatible) by using artificial saliva described by Gal et al.[23] In the present study, this medium was modified to substitute 0.5832 gm/l for 1.0 gm/l of NA_2SO_4, to promote the growth of *Desulfovibrio* spp. (MASM). However, the ASM and MASM were not adequate for the cultivation of *Desulfovibrio* spp., even with the steel samples present in our test groups, and they did not maintain growth.

Despite the loss of SRB growth activity using the ASM and MASM, analyzed on the 15th day by sub-culturing, the previous images of this assay indicated the presence of small pits of corrosion on the surface (G6 and G9) and on the border (G5) of the test samples and two pits of greater dimensions in only one test sample (G8). When we compared this data with data of the corrosive potential evaluation of the culture media, where there was no change in the samples when immersed only in ASM (G2); it was possible to observe that even with the inability of the ASM and MASM to maintain growth activity of the species *D. desulfuricans* and *D. fairfieldensis*, these bacteria were capable of degrading the metal present during the short period in which the bacteria remained viable on the media.

In the final assay, the images suggested a more intense corrosion when compared to the images from groups

G12 (ASM inoculated with *D. fairfieldensis*), G13 (ASM inoculated with *D. desulfuricans*), G14 (MASM inoculated with *D. fairfieldensis*) and G15 (MASM inoculated with *D. desulfuricans*) with the images from groups G5, G6, G8, G9 and G7. This variation of corrosion intensity may correlate with the type of technique applied for the inoculation of SRB in ASM and MASM. In group G7, where corrosion did not occur, *D. fairfieldensis* was inoculated directly onto original culture medium (MPECM) to MASM. While test samples in groups G5, G6, G8 and G9 showed some points of corrosion, the SRBs were diluted in a reducing solution for anaerobic bacteria before inoculation in the ASM and MASM. Similarly, test samples from groups G12, G13, G14 and G15 had images suggesting more intensive corrosion, where the SRBs underwent a bacterial cell washing process before inoculation of bacteria in the indicated media. In this manner, it appeared that the bacterial cell washing process totally eliminated modified Postgate E residues and bacterial products present in the medium before the inoculation of bacteria in the groups, possibly promoting the bacteria to perform more intense corrosion.

The present study also evaluates the corrosion of steel constituting the endodontic files when immersed in MPECM. When compared with all other groups, the test samples from G10 (MPECM inoculated with *D. fairfieldensis*) and G11 (MPECM inoculated with *D. desulfuricans*) presented with images most suggestive of corrosion. This finding can be explained by the fact that this medium is indicated for the growth of these bacteria, guaranteeing their viability and growth for the whole 28-day-period of incubation and assaying.[17] Therefore, the best medium for this type of biocorrosive assay seems to be modified Postgate E medium.

In relation to the morphology of the areas of corrosion and the pits of corrosion, it was not possible to establish a difference between the groups since we did not quantify the area or the volume of the metal surfaces with corrosion. However, it was noted that the majority of points of corrosion appeared on the borders of the test samples, at the interface between the metal and the resin, extending along the borders toward the center of the test sample. This finding is similar to that which occurs in the industrial area, where there is a great concentration of pits of corrosion in areas of cracks, joints and welds, the areas most subjected to the establishment of SRB biofilms, initiating biocorrosion.[28,29] To correlate this data with clinical events of file fracture in the root canal, it would be desirable that the corrosion would occur at the interface between the metal and the dentin, which could lead to or facilitate the detachment of the file from the dentine.

The use of SRB in root canals with the objective of removing fragments of fractured endodontic files is challenging, without being foolish; because even without studies about the presence of these bacteria in root canals, Lopes and Siqueira reported the presence of hydrogen sulphide as a product of decomposition in root canals.[2] Then, this finding could be an indication of the presence of SRB in these canals since their principal final product of metabolism is hydrogen sulphate. Langendijk et al isolated SRB in 0.13% of periodontal pockets related to endodontic problems, but could not isolate in 0.05% of periodontal pockets with the same clinical profile.[33] Many microorganisms present in the oral microbiota have the capacity to invade the root canals and participate in the infectious process.[34] This microbial contamination can occur through the deep periodontal pockets, enamel, cement, dentine tubules, decays, traumatic lesions or through transportation to the blood stream, or in situations where the intracanal dressing or filling is exposed to the oral environment.[34,35]

CONCLUSION

The results observed in this study demonstrated that both the environmental strain of *D. desulfuricans*, like the oral species isolated from the SRB (*D. desulfuricans* and *D. fairfieldensis*) were capable of promoting biocorrosion of the endodontic files. This biocorrosion occurred preferentially in border areas and in areas with pre-existing structural defects. Apart from this, the artificial saliva culture media and modified artificial saliva media were inadequate for the cultivation of *D. fairfieldensis* and *D. desulfuricans*, with the modified Postgate E medium being the most suitable for this process. New *in vitro* experiments will be performed with these microorganisms, such as cytotoxicity tests, biocorrosion tests of the endodontic file fragments inside the root canal and its interaction with the dentin wall, with the objective of better understanding the behavior of this group of bacteria.

CLINICAL SIGNIFICANCES

This study describes the initial development of a biopharmaceutical to facilitate the removal of endodontic file fragments from root canals, which can be successful in endodontic therapy in order to avoiding parendodontic surgery or even tooth loss with such events.

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