Biocorrosive behavior of sulphate-reducing bacteria in kerr endodontic files: Determination of the corrosion

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Abstract

Aims: This study determined the corrosion rate by mass loss caused by oral strains of sulphate-reducing bacteria (SRB) in Kerr endodontic files (KF), aiming the development of a biopharmaceutical that facilitates the removal of endodontic limb fragments from root canals.

Materials and Methods: Nine new KF were analyzed after immersion in the modified Postgate E culture medium inoculated with Desulfovibrio desulfuricans oral (84 days), Desulfovibrio fairfieldensis in the consortium (84 days) and environmental D. desulfuricans (119 days).

Results: Optical microscopy revealed corrosion suggestive areas in all files submitted to immersion in SRB cultures, presenting a statistical difference (P < 0.05) between the samples environmental D. desulfuricans and KF control and between oral D. desulfuricans and KF control. Epifluorescence microscopy revealed an active SRB biofilm over the entire metal surface of the KF, as evidenced by the SYTO® 9 fluorophore.

Conclusion: SRB were capable of promoting biocorrosion in Kerr type endodontic files, but with low rate.

Keywords: Bacteria; Desulfovibrio; endodontic files; oral microbiology; sulfate-reducing

INTRODUCTION

The complex anatomy of the root canal system, varying in shape and size, limits the success of endodontic treatment, which becomes even more complex and uncertain if an endodontic file fracture occurs.[1] 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.[2]

To remove this metal fragment, various techniques and maneuvers are employed during treatment, with successful fragment removal in most cases.[3,4] However, no technique is completely safe since perforations, destruction, or reduction in the resistance of the tooth root can occur.[5]

It is possible to consider the repeated corrosion of an endodontic file fragment within the root canal as a process that facilitates 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.[6]

SRB is 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, Desulfo monas, Desulfotomaculum, Desulfolobus, Desulfobacter, Desulfococcus, Desulfosarcina, Desul fonema, etc. They can be found in the environment, soil, freshwater, and salty marshes or in the human body, mainly in the intestinal microbiota, where the species Desulfovibrio desulfuricans can be frequently detected.

The material proposed in this study is BACCOR, a biopharmaceutical product under development with endodontic use as an aid in fractured endodontic file removal from the root canal. Previous studies have demonstrated that D. desulfuricans and Desulfovibrio fairfieldensis can promote biocorrosion of the steel constituent of endodontic files, and also, demonstrating the biocompatibility of the inoculation vehicle from cytotoxicity assays.

The objective of this study was to determine the corrosion rate caused by oral D. desulfuricans, D. fairfieldensis in the consortium and environmental D. desulfuricans in Kerr endodontic files (KF) and to analyze the changes caused by these strains on the metallic surfaces of KF, aiming the development of a biopharmaceutical (initially called BACCOR-F and BACCOR-D) that facilitates the removal of endodontic limb fragments from root canals.

**MATERIALS AND METHODS**

Nine KF No. 30, 31 mm (K-File colorinox®; Dentsply Maillefer; REF: A012D03103000, Lot: N33776580) brand new, were used. Each file was weighed in an analytical and precision electronic scale (Cubis MSA, Sartorius), autoclaved for 15 min at 121°C and kept in an oven at 50°C for 24, 48 and 72 h for later re-weighing at the end of each period. In each period, the files were weighed in triplicate. All nine files were divided into three different cell cultures using Postgate E-modified culture medium, oral D. desulfuricans, D. fairfieldensis in consortium, and environmental D. desulfuricans [Table 1]. The first two SRB samples were isolated from human saliva and the last isolate from the strains bank of the Laboratory of Biocorrosion and Biodegradation of the National Institute of Technology. Within a laminar flow chamber, a 0.1 ml aliquot of positive bacterial growth culture of the different SRB strains was inoculated into a modified Postgate E culture medium.

To analyze the characteristics of the SRB biofilm, a new Kerr file No. 30, 31 mm (K-File colorinox®; Dentsply Maillefer; REF: A012D03103000, Lot: N33776580) was added in a culture of environmental D. desulfuricans in modified Postgate E culture medium [Table 1].

**Immersion assays**

After the initial weighing analyzes, each file was individually immersed in the recently inoculated bacterial culture. The manipulation for this assay was carried out in a laminar flow chamber, and after immersion, the container was purged, sealed and incubated at 30°C in a B.O.D. incubator (Chemistry-BODQ-315M) for 84 days (2016 h) for the oral D. desulfuricans and D. fairfieldensis and 119 days (2856 h) for the environmental D. desulfuricans group [Table 1].

**Determination of mass loss**

After the incubation period, the files were incubated at 50°C for 24, 48 and 72 h for weighing in an analytical and precision electronic scale at the end of each period. In each period, the files were weighed in triplicate.

After obtaining the measurements of mass loss of the endodontic files, it was possible to obtain the corrosion rate by applying the following equation: corrosion rate = (K × W) / (A × T × D) [2] (K = Corrosion constant in pm/s [2.78 × 106 pm/s]; T = exposure time in hours; A = surface area in cm²; W = mass loss in grams; D = density in g/cm³ [7.94 g/cm³]).

**Microscopy**

Subsequently, all files were observed in an Optical Microscope 50–1000x O Bx51 (Olympus) associated with Analysis Docu Software (Olympus Soft Imaging Solutions), and a Kerr 31 mm endodontic file (K-File colorinox®; Dentsply Maillefer; REF: A012D03103000, Lot: n33776580) new, without bacterial exposure, as a control. Five different fields of each endodontic file were evaluated, measuring its largest axis, for suggestive of corrosion.

The data obtained in the measurements of the areas with corrosion indication were analyzed by the one-way ANOVA test with Tukey post hoc test, using graph Pad Prisma 5 software. The level of significance for this analysis was 5% (P < 0.05).

| Groups | Cultivation time | Testing | Microscopic analysis |
|--------|------------------|---------|----------------------|
| D. desulfuricans (oral strain) (n=3) | 84 days | Mass loss | Corrosion analysis using optical microscope |
| D. fairfieldensis (in consortium) (n=3) | 84 days | Mass loss | Corrosion analysis using optical microscope |
| D. desulfuricans (environmental strain) (n=3) | 119 days | Mass loss | Corrosion analysis using optical microscope |
| Kerr endodontic file (control) (n=1) | 119 days | - | Corrosion analysis using optical microscope |
| D. desulfuricans (environmental strain) (n=1) | 119 days | - | Biofilm analysis using epifluorescence microscopy |

B. desulfuricans: Desulfovibrio desulfuricans, D. fairfieldensis: Desulfovibrio fairfieldensis

Table 1: Distribution of groups according to the analysis for each sample
In parallel, an endodontic file Kerr n° 30 was observed by epifluorescence microscopy, immersed in a modified Postgate E culture medium with environmental *D. desulfuricans*. The confocal laser scanning microscope LSM-710 (ZEISS) was used, and the images obtained were analyzed and processed with the software ZEN 2009 (ZEISS).

**RESULTS**

**Corrosion rate by mass loss**

The calculation of the corrosion rate by mass loss of the endodontic files revealed a low corrosion rate [Table 2], according to the corrosion rate categorization of ASTM G1[12] and NACE International[13] [Table 3].

**Microscopy**

Figure 1 shows the analyzes in the Optical Microscope 50–1000x Olympus Bx51 and areas of corrosion suggestion along the metal surface of all the endodontic files submitted to immersion in SRB cultures. The KF control showed areas of minor suggestive corrosion, revealing in most of the images a metallic surface with no corrosion damages, only with machining slots from the production process.

In comparison between the groups, a statistically significant difference was observed between the environmental *D. desulfuricans* and control file samples and between oral *D. desulfuricans* and control file [Figure 2].

Epifluorescence microscopy revealed an active SRB biofilm on the metal surface of the endodontic file [Figure 2]. It was possible to demonstrate the cellular activity of environmental *D. desulfuricans* in prolonged culture time, 119 days. Areas with higher fluorescence intensity were also observed, suggesting a higher cell concentration, indicating a structured biofilm in these areas, which would be facilitator since the formation of biofilm areas would also help the formation of corrosion pits, as described in the theory of cathodic depolarization.[8,9]

**DISCUSSION**

After contact of the liquid medium with the metal, a film of organic molecules occurs and modifies the wetting and the distribution of charges on the metallic surface, facilitating the adherence of the microorganisms present in the liquid. The microbial cells present on the metallic surface start the process of colonization and production of the extracellular polymeric matrix, forming the biofilm.[8,9,14] However, the biofilm is directly influenced by the type of material[15] and by the presence of surface roughness, becoming a facilitator for fixing these bacteria and establishing the surface biofilm.[16] Once formed, biofilms can modify the metal/solution interface to induce, accelerate and/or inhibit the anode or cathode process that controls the biocorrosion reaction.[14]

The application of CLSM allows the visualization of totally hydrated biofilm samples to investigate their development, maintaining its integrity.[17] Okabe et al.[18] reported the use of CLSM with the fluorophore TRITC in the analysis of the spatial distribution of SRB in aerobic biofilms of 40 days, demonstrating the presence of this microorganism and mineral compounds. Purish et al.[19] reported the analysis of a 90 days biofilm on the metal surface, with an initial inoculum of 10% *Desulfovibrio* sp. As in our study, the authors described a structured biofilm surface, forming microbial aggregates and interstitial voids.

The importance in demonstrating, through these tests, the presence of the biofilm lies in the fact that the formation of the SRB biofilm on the metal surface will directly influence the corrosion rate, altering the transport of chemical elements, favorably or unfavorably, facilitating the removal of a protective coat over the metal surface inducing differential aeration and an irregular distribution of the biofilm.[20]

Different from the previous study of the same group on the biocorrosive action of the SRB in endodontic files,[10] the current investigation associates an analytical technique with the mass loss method, to obtain an intensity of the SRB biocorrosive process, in endodontic files.

Previous studies have evaluated corrosion by mass loss in different dental metal alloys.[21] Sutow et al.[21] for example, evaluated the dilution rate in the mass loss in Thermafil Endodontic Obturator Carriers of stainless steel AISI 302 when immersed in 50 ml of 0.9% NaCl solution. At the end of the 4-week period, a low mass-loss rate was identified, being iron the main released element (from 0.55 to 3.67 µg/cm²/4 weeks), followed by nickel, chromium, molybdenum, manganese, and copper. These results were confirmed by the absence of corrosion pits when observed by SEM. Although these results refer to abiotic corrosion, the above data showed greater mass loss when compared

**Table 2: Corrosion rate by weight loss**

| Groups | Corrosion rate (mm/year) | Time/h |
|--------|--------------------------|--------|
| *D. desulfuricans* ambiental | 0.0003095 | 2856 |
| *D. desulfuricans* oral | 0.0002529 | 2016 |
| *D. fairfieldensis* | 0.0002165 | 2016 |

**Table 3: Qualitative categorization of the corrosion rate**

| Category | Average corrosion rate (mm/year) |
|----------|----------------------------------|
| Low      | <0.025                           |
| Moderate | 0.025–0.12                       |
| High     | 0.13–0.25                        |
| Severe   | >0.25                            |
to biocorrosion. However, when comparing the metallic surfaces, the authors demonstrated an intact surface, while in the biocorrosion, it was possible to reveal the formation of corrosion pits, which would be acceptable to facilitate the removal of a fractured endodontic file inside the root canal.

The results of the biocorrosion rate presented here were inexpressive when correlated with the immersion time of the endodontic files used. However, Isa et al.\textsuperscript{[22]} demonstrated the highest activity of SRB in anaerobic reactors between 11 and 24 days, decreasing the production of sulfur between 54 and 63 days. This fact can be explained by the supersaturation of iron sulfide in the medium, the main metabolite resulting from the SRB biocorrosion, as described by Jhobalia et al.\textsuperscript{[23]}, who verified the sudden drop of corrosion in steel coupons when the solution was super saturated with iron sulfide, remaining stable. Throughout the assay, the authors reported that increased sulfide concentration has decreased the SRB growth rate and corrosion rate. Thus, the low corrosion rate may be due to the long period of the 84 and 119-day immersion time.

**Figure 1:** Images of Kerr endodontic files in optical microscope: Control group showing intact surface (a) and areas of corrosion suggestion (b) indicated by red arrow (b1); Group after immersion in media modified Postgate E with environmental \textit{Desulfovibrio desulfuricans}, indicating areas of corrosion by white and red arrows. Areas indicated by red arrows in the images c and d and enlarged in the images c1 and d1, respectively; Group after immersion in media modified Postgate E with \textit{Desulfovibrio fairfieldensis}. Areas indicated by the red arrows in the images e and f and enlarged in the images e1 and f1, respectively; Group after immersion in media modified Postgate E with oral \textit{Desulfovibrio fairfieldensis}. Areas indicated by red arrows in g and h images and enlarged in g1 and h1 images, respectively.

**Figure 2:** The graph presents the diameter of the areas of corrosion suggestion undergoing immersion test with sulfate-reducing bacteria and control sample. Analysis in confocal laser scanning microscopy (a and b): Active biofilm on the metal surface of the endodontic file immersed in media modified Postgate E with environmental \textit{Desulfovibrio desulfuricans}. 

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\textsuperscript{[22]} Isa et al. \textsuperscript{[23]} Jhobalia et al.
cultures of the present study, which leads to the need in future tests for periodic exchanges of the culture medium, the SRB inoculation vehicle, to reduce the concentration of iron sulfide for higher efficiency of corrosion rate by SRB.

The main concern regarding the formation of the biofilm on metallic surfaces by *Desulfovibrio* spp. has also been focus in dental implants, being demonstrated the formation of a structured biofilm of *D. fairfieldensis* after 24 h incubation. However, Jorand *et al.* [24] demonstrated that a 40-day maturity biofilm had a comparable activity of a 24-h biofilm and that the cells entered the stationary phase near 25 days of growth. [24] Thus, these results may justify the low corrosion rate in the 84 and 119-day trials, indicating an optimization of the biocorrosion being aware of the SRB growth curve.

**CONCLUSION**

SRB are capable of promoting biocorrosion in Kerr type endodontic files, maintaining a biofilm active for a long period. However, this biocorrosion does not appear significant in relation to the corrosion rate due to mass loss in the periods tested, differing from the superficial changes in areas of suggestive corrosion found in the microscopy analysis. Further analyzes are required to define the bio-corrosive strength of SRB in Kerr-type endodontic files.

**Acknowledgments**

This work was supported by the Coordination for the Improvement of Higher Education (CAPES), Research Support Foundation of the State of Rio de Janeiro (FAPERJ), National Council for Scientific and Technological Development (CNPq).

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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