

A novel bioactive glass-ceramic for treating dentin hypersensitivity

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Abstract: Dentin hypersensitivity (DH) is a painful response to stimulus applied to the open dentinal tubules of a vital tooth. It's a common oral condition, however, without an ideal treatment available yet. This work evaluated *in vitro* the effect of micron-sized particles from a novel bioactive glass-ceramic (Biosilicate) in occluding open dentinal tubules. A dentin disc model was employed to observe comparatively, using scanning electron microscopy (SEM), dentinal tubule occlusion by different products and deposition of hydroxyl carbonate apatite (HCA) on dentin surface by Biosilicate, after a single application: G1 - Dentifrice with potassium nitrate and fluoride; G2 - Two-step calcium phosphate precipitation treatment; G3 - Water-free gel containing Biosilicate particles (1%); G4 - Biosilicate particles mixed with distilled water in a 1:10 ratio; all of them after 1, 12 and 24 hours of immersion in artificial saliva. Fourier transform infrared spectroscopy (FTIR) was performed to detect HCA formation on dentin discs filled with Biosilicate after 2 minutes, 30 minutes and 12 hours of immersion in artificial saliva. SEM showed a layer of HCA formed on dentin surface after 24 hours by G4. G1, G2 and G3 promoted not total occlusion of open dentinal tubules after 24 hours. FTIR showed HCA precipitation on the dentin surface induced by Biosilicate after 30 minutes. The micron-sized particles from the bioactive glass-ceramic thus were able to induce HCA deposition in open dentinal tubules *in vitro*. This finding suggests that Biosilicate may provide a new option for treating DH.

Descriptors: Biocompatible Materials; Dentin Sensitivity; Dentin.

Introduction

Dentin hypersensitivity (DH) is a common oral condition, but an ideal product or protocol for its treatment does not exist, and active management is a challenge. DH is caused when the fluids within the dentinal tubules are subjected to changes (thermal, mechanical, osmotic). The movement in the fluids stimulates a nerve receptor sensitive to pressure, which leads to the transmission of the stimuli. Consequently, dental products proposed to treat DH seek to interrupt the pulp neural response of pain and/or to block the sensitive mechanisms through occlusion of the open dentinal tubule.¹⁻²

A fully crystallized bioactive glass-ceramic ($P_2O_5-Na_2O-CaO-SiO_2$) named Biosilicate, which has been developed by a multidisciplinary research group, has been proposed to treat DH by hydroxyl carbonate

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Received for publication on Jun 16, 2010
 Accepted for publication on Aug 07, 2010

apatite (HCA) deposition in open dentinal tubules.³ Bioactive glasses and glass-ceramics are widely recognized as one of the best clinical choices to improve bone regeneration,⁴ and the similarity of composition between bone, dentin and enamel led to the assumption that bioactive glasses and glass-ceramics could also be efficient for the regeneration of enamel and dentin. Indeed, the hypothesis was that glass-ceramics could treat DH by providing permanent occlusion of the open dentinal tubules through *in situ* deposition of a HCA-bonded layer.⁵⁻⁶

The first experiments with Biosilicate corroborated its bioactivity.⁷⁻⁸ In addition, crystallization has been shown to significantly change the fracture characteristics of glass, which provided less-sharp, less-abrasive particles.⁹ Therefore, crystallization hypothetically results in two advantages:

- i. the dull particles can be safely added to any kind of formulation to be used in the oral environment, and
- ii. the particles can be easily inserted into dentinal tubules because there are no edges to deflect them away from the orifices.

The aim of this study was to test the hypothesis that Biosilicate could be an effective desensitizing agent for the treatment of DH. To test this hypothesis, we analyzed *in vitro* Biosilicate comparatively against commercial materials and evaluated three parameters:

- i. dentinal tubule occlusion provided by the products tested and deposition of HCA triggered by Biosilicate in open dentinal tubules;
- ii. the effect of two different vehicles for the incorporation of Biosilicate particles; and
- iii. the reaction of Biosilicate formulations on dentin discs after different time periods.

Materials and Methods

The research protocol was approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, USP, Brazil (process # 2003.1.654.58.7).

Preparation of the desensitizing agents based on Biosilicate

The desensitizing methods tested in this study, including product concept, desensitizing agent and

mechanisms of action are shown in Table 1. To simulate home-use and professional-use products, we sought to establish the simplest and safest mode of application because safety and ease of application are two of the desirable characteristics of desensitizing products. For simulation of home-use products, a water-free gel based on Carbopol and glycerin (Sigma Chemical Co., Saint Louis, USA) was used as a vehicle for the Biosilicate particles. The water-free gel containing 1% Biosilicate was formulated at the School of Pharmaceutical Sciences of Ribeirão Preto, USP, Brazil, and inserted into a sterilized (7 g) dentifrice tube (Embagel®, Pharmaceutical tube, code 211, São Paulo, SP, Brazil). For simulation of the professional-use products, the Biosilicate particles were mixed with distilled water immediately before application. A 1.5-mL tube (Microtubes safe-lock, Eppendorf® Brazil, São Paulo, SP, Brazil) containing 0.15 mg of Biosilicate powder was filled with 1.35 mL of water, which provided a 1:10 mixture of Biosilicate powder in distilled water.

Preparation of dentin discs

This study employed the dentin disc model with a *strict control* methodology, which has been described in the literature.⁵ A total of 40 dentin discs were obtained and stored in a vial with 100 ml of distilled water. Each disc was fractured at the center using wire cutters to provide test and control halves. Both parts of each disc were stored together in a 1.5-mL tube containing artificial saliva.

Five control and experimental halves were randomly assigned to four groups (Table 1). Immediately before the application of the proposed materials, the control and test halves of each disc were etched in 6% citric acid for 2 minutes, washed with distilled water for two minutes and stored in labeled tubes containing artificial saliva. At no time during the procedures were discs allowed to dry.

Application of the product to the dentin discs

During product application, the test disc was pulled out of the 1.5-mL tube, and the excess artificial saliva was removed by briefly pouring the dentin disc onto a piece of filter paper. Humidity was

Table 1 - The experimental groups of the study, including product concepts regarding their mode of use, desensitizing agents and proposed mechanisms of action.

Group	Product concept	Desensitizing agent	Proposed mechanism of action
G1 - Desensitizing dentifrice (Sensodyne®, GSK Brasil, Rio de Janeiro, RJ, Brasil).	Home-use	5% potassium nitrate; 1187 ppm of MFP (active fluoride)	Depolarizing effect on neural conduction of pain
G2 - Desensitizing solutions (Sensi Kill®, DFL, Rio de Janeiro, Brasil).	Professional use	Solution 1: phosphate dipotassium, sodium fluoride, methylparaben and distilled water. Solution 2: calcium chloride, sodium benzoate and distilled water.	Calcium phosphate deposition on the dentin surface - tubule occlusion
G3 - Experimental desensitizing gel Vitrovita®, São Carlos, SP, Brasil.	Home-use	Fully crystallized bioactive glass-ceramic (Biosilicate)	HCA layer formation on dentin surface - tubule occlusion
G4 - Experimental desensitizing solution Vitrovita®, São Carlos, SP, Brasil.	Professional use	Fully crystallized bioactive glass-ceramic (Biosilicate)	HCA layer formation on dentin surface - tubule occlusion

preserved in the test discs, and control discs were maintained in the 1.5-mL tubes with artificial saliva for either 1, 12 or 24 hours. The materials were applied to test discs positioned on a sterile glass surface as follows:

- G1: Approximately 0.15 mL of toothpaste was poured onto the test disc, and the product was dispersed over the dentin surface for 30 seconds using a micro-applicator (Microbrush® Tube Series, Fine size, MFA 400, Grafton, USA).
- G2: Solution 1 was rubbed on the test disc for five seconds and kept moistened with the solution for 30 seconds. With another micro-applicator, solution 2 was rubbed on the same test disc for two seconds and kept moistened for ten seconds in accordance with the manufacturer's instructions.
- G3: One drop (about 0.2 mL) of gel from a tube was dropped on the test disc. The gel was gently dispersed on the dentin surface using a micro-applicator for 30 seconds.
- G4: Approximately 0.15 mL of the solution was gently applied to the test disc with a micro-applicator for 30 seconds.

After the application of each product for its respective duration, each test disc was gently washed with distilled water for 30 seconds and stored in a tube containing 1.5 mL of artificial saliva for either 1, 12 or 24 hours. The discs remained in artificial saliva for the mentioned periods, and after that, the

control and test halves were pulled out of the 1.5-mL tube and air dried in a desiccator (Eikonal do Brasil, São Paulo, SP, Brazil) at 36°C for 1 week. The discs were then mounted on aluminum stubs and photographed with an SEM (FEG2000, Philips, Oregon, USA).

FTIR analysis

To investigate the conversion of the Biosilicate into HCA on the dentin surface, dentin discs were analyzed by a FTIR spectrometer immediately after product application as well as 2 minutes, 30 minutes and 12 hours after application and immersion in artificial saliva. Pure Biosilicate and dentin control discs were also analyzed with the FTIR spectrometer.

Results

Scanning electron micrographs

The experimental and control discs had comparable diameters and showed a similar pattern of tubule distribution and orientation. Figure 1 shows an overall view of the micrographs taken from the dentin test discs after different periods of immersion in artificial saliva. After 1 hour of immersion in saliva, the front surface of the open dentinal tubules did not show the same pattern. Indeed, some particles could be observed on the dentin surface of the G1 discs (image a) but not inside the dentinal tubules, which was probably due to the size and morphol-

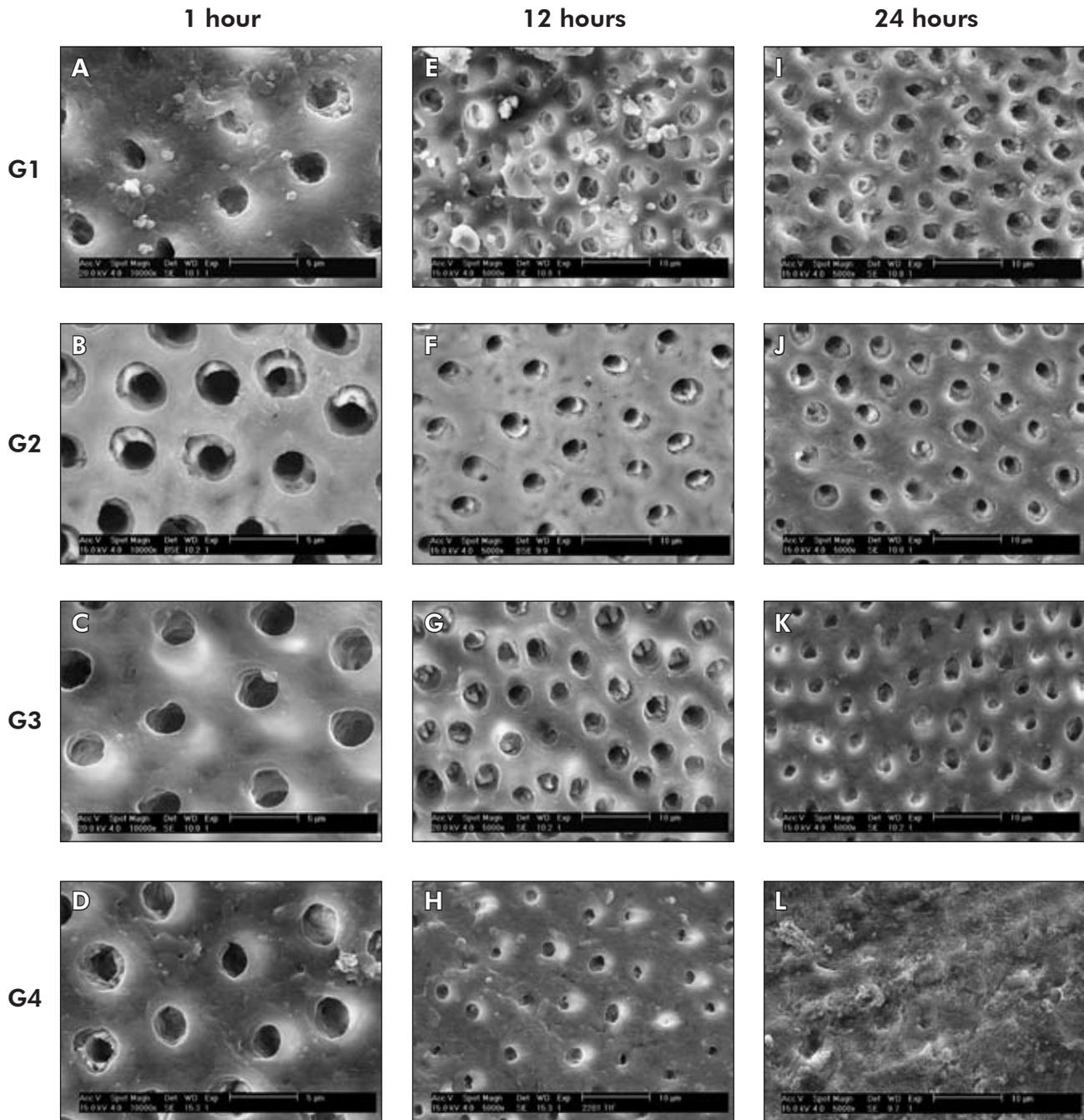


Figure 1 - SEM micrographs of the test discs at different durations of immersion in artificial saliva. The images on the left, middle and right columns show dentin discs where the products were applied after 1, 12 and 24 hours of immersion in artificial saliva, respectively. The images from the top to the bottom rows show dentin discs allocated in accordance with the experimental group: G1, G2, G3 and G4, respectively.

ogy of the particles contained in the dentifrice formulation. Few or no particles can be observed on the surfaces of the G2 (image b), G3 (image c) or G4 discs (image d), probably due to the fact that G2 has a liquid constitution and Biosilicate has micron-

sized particles dispersed in the distilled water or gel, which were possibly inserted inside the dentinal tubules by gentle application. After 1 hour of immersion, the G2 discs (image b) showed dentinal tubules with diminished diameters, and the G3 (image c)

and G4 (image d) experimental discs showed little (image c) to no tubule occlusion (image d). After 12 hours, the G1 discs (image e), displayed a large number of particles on the dentin surface. The G2 discs at 12 hours (image f), however, were not changed compared with the G2 discs after 1 hour of immersion (image b). In the G3 discs, more particles were observed inside the dentinal tubules at 12 hours (image g) than at 1 hour (image c). Interestingly, a noticeable difference was observed in the dentinal tubule diameter of G4 discs at 12 hours compared with 1 hour (image h).

After 24 hours of immersion, the G1 disc surface showed some open dentinal tubules and a weak pattern of tubule occlusion (image i). Compared with the G2 discs at 12 hours (image f), the G2 discs at 24 hours (image j) showed a small decrease in the dentinal tubule diameter. Compared with the findings in G3 discs at 12 hours (image g), tubule occlusion increased in the G3 discs after 24 hours (image k). Interestingly, the G4 discs showed total obliteration of the dentinal tubules after 24 hours of immersion in artificial saliva (image l).

Fourier transformed infrared analysis

The results of FTIR analysis plotted in Figure 2 show the Biosilicate reactions incorporated on

a dentin surface until complete conversion into a HCA layer. The mechanism of HCA formation on a monolithic glass-ceramic was described by Peitl *et al.*,¹⁰ and Biosilicate powder undergoes the same steps. The line a shows pure Biosilicate without reaction and the line c shows pure dentin. After 2 minutes in artificial saliva we observe, at the line e, a mixture between the peaks from dentin and Biosilicate. The main spectral peak assignments for the molecular vibrations of Biosilicate are observed at 460, 536, 930 nm, and at 1124, 602, 574 nm for dentin. The double peaks at 602 and 574 nm are the most important P-O crystal vibrational bend mode associated to HCA. We clearly observe these peaks on pure dentin. Increasing time reactions to 30 minutes in the solution (line d), we observe the phosphate peaks, 602 and 574 nm, develop and the Biosilicate decrease as 460 and 930 nm. This is thus experimental evidence for the formation of HCA on Biosilicate surface. We conclude from these spectra that the Biosilicate formed a thin layer of HCA on dentin. Finally, after 12 hours (line b) we cannot observe the Biosilicate peaks, but only the identical spectra showed by pure dentin. Therefore, at this time it is not possible to distinguish the substrate and dentine from reacted Biosilicate.

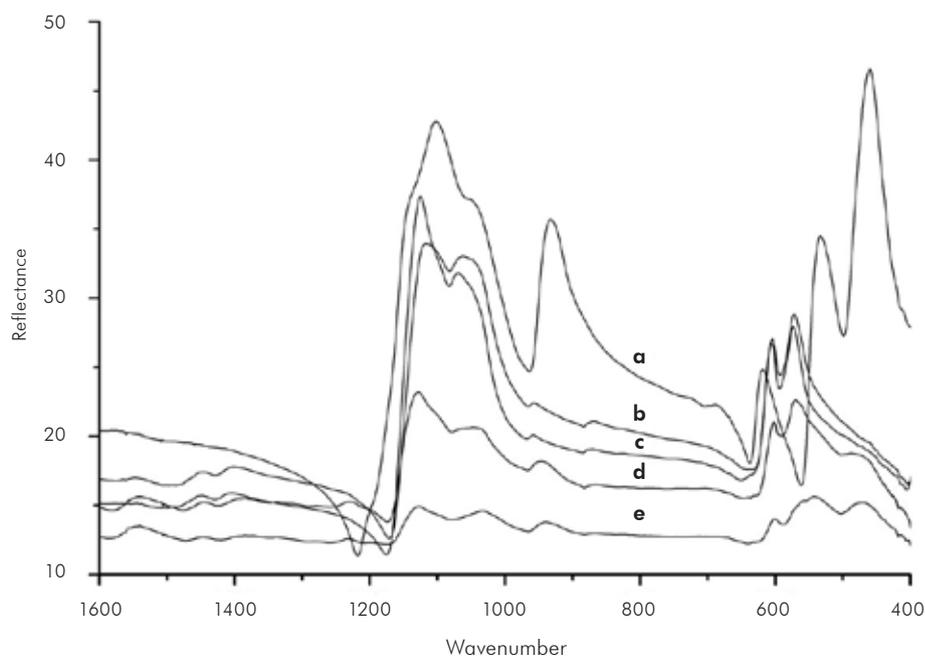


Figure 2 - FTIR spectrum. Line **a**: pure Biosilicate; line **b**: Biosilicate on dentin disc over 12 hours in artificial saliva; line **c**: control dentin disc; line **d**: Biosilicate on dentin disc over 30 minutes in artificial saliva; line **e**: Biosilicate on dentin disc over 2 minutes in artificial saliva.

Discussion

Studies regarding desensitizing agents have indicated that, nowadays, the treatments currently used to block the sensitive mechanisms of DH pain could be improved to obtain easy, fast, non-invasive, durable relief of patient discomfort.

The first aim of this study addressed the dentinal tubule occlusion of the tested products as well as the deposition of HCA triggered by Biosilicate in open dentinal tubules. Although only the treatment used in G4 triggered total occlusion, the treatments used in G1, G2 and G3 also promoted a decrease in the diameter of the dentinal tubules. In G1, a widely used desensitizing dentifrice was used. Although one study¹¹ showed considerable dentinal tubule occlusion by desensitizing dentifrices, in this study dentin surfaces were brushed for over two minutes twice a day for seven days, which probably created smear layer deposits in the tubules. In G2, the film of mineralized deposit on the dentin surface was not observed at 24 hours (dissolution or dislodgment were supposed) as it was for G4.

We also examined the ability of Biosilicate to occlude open dentinal tubules by inducing HCA formation. The FTIR analysis showed that HCA was formed after a reaction time of 30 minutes. It is important to note that the test products were only applied once in the present study, and they were gently applied with a micro-applicator for a few seconds to ensure that the materials covering and inside the dentinal tubules were exclusively the products tested and not a smear layer. The fine particle size was important to allow a faster reaction and deposition of HCA inside the dentinal tubules and on the dentin surface.¹² Considering the properties of bioglasses and the images from this *in vitro* investigation, it is possible that the micron-sized particles of Biosilicate, which ranged from 0.1-10 μm , became smaller as the contact time with the liquid increased. The present study also addressed the interplay between Biosilicate particles and two different vehicles. Related to this, Biosilicate mixed with distilled water (1:10) performed much better than Biosilicate in the gel (1:100). The difference shown by the same particulate bioactive material in occluding open dentinal tubules and covering the dentin surface was

probably due to the percentage of incorporation of Biosilicate into the gel, which was much lower than the amount of powder mixed into distilled water. In addition, the cross-linked structure of the gel may have prevented Biosilicate particles from escaping. Only 1% Biosilicate was incorporated into the water-free gel because we were trying to simulate a daily home-use product. The formulation of the Biosilicate mixed with distilled water, however, was meant for professional use, and the ratio was established at 1:10. This difference in the amount of particles had an important effect on tubule occlusion, which has also been suggested by Lee *et al.*⁶

The present study also investigated how Biosilicate formulations react on dentin discs after different durations. The best performance of the bioactive material occurred after 24 hours of immersion in artificial saliva. This suggests that a bonded layer of HCA formed on the dentin surfaces, which resisted the dislodgment that immersion promotes. This hypothetically permanent and difficult-to-remove obliteration promoted by the biomaterial in the substrate surfaces (dentin surface and tubules) was corroborated by a recent study¹³ that evaluated the biomechanical behavior of the tissue formed in tibial bone defects. Because we investigated dentin discs immersed in artificial saliva for up to 24 hours, we were able to observe how a single application of DH products affected the dentinal tubules and dentin disc surfaces over time.

The present study produced several interesting findings:

- i. the products tested showed different patterns of dentinal tubule occlusion, and deposition of HCA triggered by Biosilicate occurred in open dentinal tubules;
- ii. the type of vehicle and the amount of Biosilicate particles played an important role in desensitizing formulations containing Biosilicate (i.e., different patterns of interaction between dentin discs and Biosilicate particles were observed for G3 and G4); and
- iii. the time needed for a homogeneous layer of HCA to cover the dentin surface was 24 hours. Taken together, these results provide a preliminary con-

firmation of the research hypothesis that micron-sized Biosilicate particles are capable of occluding open dentinal tubules and could be used in DH treatments.

Further *in vitro* investigations with different ratios of particles incorporated into water-free gels are needed to determine the best proportion of Biosilicate in home-use products. In addition, other studies should test the bonded character of the HCA layer and investigate the hydraulic conductance in the dentinal tubules occluded with the Biosilicate particles. A six-month clinical study was carried out because of these *in vitro* results. That study confirmed the results of the present *in vitro* experiments (i.e.,

the efficacy of Biosilicate) and is being described in a forthcoming paper.

Conclusions

Micron-sized bioactive glass-ceramic (Biosilicate) particles were able to induce HCA deposition in open dentinal tubules, which suggests that the material could provide a new option for treating DH.

Acknowledgements

We would like to thank the FAPESP (contract number 04/05133-0 and 07/08179-9), and the CNPq (contract number 0400604/2004-3) for financial support of this work.

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