REVISTA DE ODONTOLOGIA DA UNESP

ORIGINAL ARTICLE

Rev Odontol UNESP. 2016 Nov-Dec; 45(6): 339-343
Doi: http://dx.doi.org/10.1590/1807-2577.12216

Evaluation of the dentin changes in teeth subjected to endodontic treatment and photodynamic therapy

Avaliação das alterações morfológicas de dentes submetidos ao tratamento endodôntico e a terapia fotodinâmica

Mariane Floriano Lopes Santos LACERDA[a], Carolina Oliveira de LIMA[a], Gabriel Pinheiro LACERDA[a], Celso Neiva CAMPOS[a]*

[a]UFJF – Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil

Resumo

Introdução: A PDT antimicrobiana é um eficiente método coadjuvante na desinfecção do sistema de canais radiculares, o que torna importante o estudo sobre a possibilidade de alterações morfológicas e permeabilidade provocadas pelo laser de diodo na estrutura dentinária radicular. **Objetivo:** A proposta deste estudo foi investigar as alterações morfológicas e percentual de infiltração apical radicular após o uso do laser diodo. **Material e método:** Quarenta dentes monorradiculares foram instrumentados pelo sistema rotatório Protaper e irrigados com NaOCl 5,25% e EDTA 17%. Depois, foram divididos aleatoriamente em: G1: não receberam PDT (controle); G2: receberam pré-irradiação com fotossensibilizador azul de toluidina e irradiação com laser diodo de AsGaAl. Em seguida, 10 dentes de cada grupo foram analisados por MEV quanto as alterações morfológicas. Os outros 10 dentes foram obturados e corados por Rodamina B para avaliar o percentual de infiltração apical. **Resultado:** Os resultados mostraram diferença significativa entre G1 e G2 (p<0,001 - qui-quadrado), verificando maior presença de debris em G1 (controle) e maior ocorrência de túbulos dentinários abertos em G2 (PDT). Erosões e *cracks* foram observadas em ambos os grupos, sem diferença estatística significativa (p>0,001). O percentual de infiltração apical foi significativamente maior em G2 que em G1 (p<0,001 – t *student*). **Conclusão:** Concluiu-se que a utilização do laser diodo promoveu a redução de *smear layer* e abertura dos túbulos dentinários, não influenciando na ocorrência de erosão, cracks e carbonização. O laser aumentou a permeabilidade da dentina apical.

Descritores: Fotoquimioterapia; laser; endodontia.

Abstract

Introduction: Antimicrobial photodynamic therapy (PDT) is an efficient adjuvant technique to promote disinfection of the root canal system. Therefore, it is important to investigate changes to dentin morphology and permeability induced by the use of diode laser on the root dentin. **Objective:** The purpose of this study was to investigate morphological changes and the percentage of apical leakage after the use of laser. **Material and method:** Forty single-rooted teeth were instrumented using rotary system and irrigated. Teeth were randomly divided in two groups: G1 - not exposed to PDT (control), and G2 - pretreated with toluidine blue photosensitizer and irradiated with AsGaAl laser diode. Ten teeth in each group were evaluated by SEM for morphological changes. The other ten teeth were filled and stained with Rhodamine B to evaluate the apical leakage. **Result:** The results showed significant difference between G1 and G2 (p <0.001 - chi-square), with greater presence of debris in G1 and higher incidence of open dentinal tubules in G2. Erosions and cracks were observed in both groups, with no statistically significant difference (p> 0.001). The apical leakage was significantly higher in G2 than in G1 (p <0.001 - Student's t-test). **Conclusion:** It was concluded that the use of low-level laser reduced the smear layer and opened the dentinal tubules. Use of laser increased the permeability of the apical dentin.

Descriptors: Photodynamic therapy; lasers; endodontics.

INTRODUCTION

Persistence of microorganisms in the root canal system (RCS) is the main cause of endodontic therapy failure owing to the presence of metabolic products and to formation of biofilm, which acts as a source of infection. These microorganisms may colonize dentinal tubules, accessory canals, isthmuses, and apical deltas, rendering their eradication difficult solely by instrumentation, irrigation, and use of intracanal medications[1].

Additionally, the host defenses or microbial agents are often unable to reach the microorganisms remaining within the RCS or in extraradicular regions[2]. In these situations, endodontic therapy requires complementary techniques to enhance the antimicrobial action and achieve decontamination.

Low-level laser irradiation has been proposed as an adjuvant to chemomechanical preparation (CMP) of the root canal in cases of infection, with the goal of increasing the efficacy of endodontic treatment. Several researchers have studied the bactericidal effect of low-level laser irradiation, in a procedure known as photodynamic therapy (PDT)[3].

In PDT, microorganisms are exposed to a light-sensitive compound and become susceptible to irradiated light, which induces a photochemical reaction that generates free radicals and singlet oxygen. This leads to rupture of bacterial cell walls and destruction of the microorganisms[4].

However, the effects of this technology on the dentin structure should be investigated, in view of the possibility that it may induce morphological changes to the dentinal tubules and to dentin permeability, which in turn could hinder the sealing ability of filling cements. If the sealing ability is impaired, reinfection of the RCS may occur, allowing the formation of new sources of infection and culminating in endodontic treatment failure[1].

With this in mind, the aim of the present study was to evaluate *in vitro*, by quantitative and qualitative analyses, the changes in permeability of dentinal tubules and of the dentin structure after PDT using a 660nm laser diode as an adjuvant to CMP.

MATERIAL AND METHOD

This study protocol was approved by the Ethics Committee of the Federal University of Juiz de Fora. Forty single-rooted extracted human teeth were selected for this study. The presence of a single canal was determined by mesio-distal and buccal-lingual digital radiographs and confirmed after conventional root canal access with diamond and Endo-Z burs (Dentsply/Maillefer, Switzerland). The working length (WL) was established by inserting a size 10 K-file (Dentsply/Maillefer S.A., Petrópolis, Rio de Janeiro, Brazil) into the canal, until its tip was visible at the apical foramen under 10X magnification. The endodontic rubber stop was placed against the edge of the tooth and the file was removed from the canal. The distance from the file tip to the rubber stop was measured on an endodontic millimeter ruler and the WL for each tooth was determined by subtracting 1mm from the total length obtained.

Following that, the teeth were instrumented by a single endodontist, using ProTaper rotary NiTi files (Dentsply/Maillefer S.A., Ballaigues, Switzerland), and following the "free tip preparation technique"[5]. A new set of files was used after eight instrumentations. Instrumentation was carried out using an X-Smart endodontic micromotor (Dentsply/Maillefer S. A., Ballaigues, Switzerland) at constant speed and torque of 300 rpm and 4.5 N/cm^2, respectively. The file sequence used was: SX, S1, S2, F1, and F2; the four latter instruments were used up to the WL. The F3 file was not used in any of the teeth, in order to prevent instrument fracture in narrow canals.

After each file, the canal was irrigated with 5 ml of sodium hypochlorite at 5.25%, using a Luer lock syringe attached to a 30-G navy tip (Ultradent, South Jordan, UT, USA). Tips were inserted as deep as possible inside each canal and the irrigant was removed by a suction tip. The irrigation procedure lasted approximately 30 seconds.

Following instrumentation, each canal was irrigated with 5 ml of EDTA at 17% and pH 7.5, which was allowed to remain in the canal for 3 minutes. After that, the canal was irrigated with 10 ml of distilled water. Canals were dried with F2 absorbent paper points (ProTaper Point – Dentsply/ Maillefer S.A., Petrópolis, Rio de Janeiro, Brazil).

Finally, each tooth was rendered impermeable by applying two layers of clear polish with a brush to the entire root surface, except for the root canal opening on its coronal portion. The interval between applications of each layer of polish was 30 minutes[6].

After these procedures, the 40 teeth were randomly divided into two experimental groups, with 20 specimens each:

Group 1: teeth subjected to instrumentation, irrigation with sodium hypochlorite at 5.25%, irrigation with 17% EDTA;

Group 2: teeth subjected to instrumentation, irrigation with sodium hypochlorite at 5.25%, irrigation with 17% EDTA and, after that, irradiation with low-level laser (PDT).

Low-level Laser Irradiation

Teeth in G2 had their root canals filled with 6 μg/mL toluidine blue stain (TBO) (Fórmula & Ação, São Paulo, Brazil) for 5 minutes, by means of a 0.33 cc insulin syringe[7].

Low-level 660 nm AsGaAl (arsenide, gallium, aluminum) laser was applied for 5 minutes using a continuous wave red laser diode unit (Photon Lase III, DMC Equipamentos Ltda., São Carlos, SP, Brazil). The laser parameters were: 600 nm wavelength, width of approximately 10nm, 100 mW power, and 12 J energy[6].

A 400 μm optical fiber was attached to the laser unit, inserted into the root canal up to the WL and the laser was activated. Then, the tip was slowly withdrawn while touching the buccal surface of the root canal wall. Afterwards, the same procedure was performed with the tip touching the palatal, mesial, and distal surfaces of the root canal wall.

Following that, the teeth were subjected to morphological analysis by scanning electron microscopy (SEM) and to evaluation of the apical leakage.

Morphological Analysis of the Root Dentin

Ten teeth from each group were randomly selected, cleaved in two fragments and examined under an SEM microscope (model JEOL JSM-T220 A) operating at 15 kV. Photomicrographs were taken under 500X and 3000X magnification. A screen with a central square measuring 20 × 20 cm was placed on the computer monitor, in order to outline the area to be analyzed. This allowed the apical third of each specimen to be observed in a standardized manner with the aid of the Meazure 2.0 Build 158 image software.

Two trained observers analyzed the fragments. They were previously calibrated by individually evaluating images with characteristics of specific morphological changes, on alternate days and times (three times a day for three days). After the third day, the observers analyzed the images together, in order to establish a consistent observation pattern of the morphological features to be scored.

Each fragment was analyzed and scores were attributed to each of the following morphological changes:

1- Presence of debris: none (0); low (1); medium (2); high (3);

2- Incidence of open dentinal tubules: none (0); low (1); medium (2); high (3);

3- Dentinal tubule erosion: absent (0); present (1);

4- Dentinal tubule cracks: absent (0); present (1);

5- Carbonized dentinal tubules: absent (0); present (1);

6- Other changes: absent (0); present (1).

Evaluation of Root Dentin Permeability

Ten teeth in each group had their root canals filled according to Tagger's hybrid technique, using ProTaper F2 gutta-percha cones (ProTaper – Dentsply/Maillefer S.A. Ballaigues, Switzerland) and endodontic sealer (Endofill). The obturating materials were compacted with a size 50 engine plugger attached to a slow-speed handpiece for 7 seconds. After root canal filling, the coronal portion of each tooth was made impermeable with clear polish, as previously described.

Teeth were immersed in 0.5% Rhodamine B dye solution (Laboratório Synth, São Paulo, SP, Brazil) in an ultrasonic bath at 37°C for 48 hours. Following that, specimens were irrigated with distilled water and dried with size F2 absorbent paper points. The roots were then sectioned lengthwise and the filling material was removed with the aid of a cotton plier. Fragments were placed on a sheet of graphing paper and photographed using a Canon T3i camera attached to a stand. The focal distance was set at 40 cm.

Morphometric analysis was carried out using the Image J software. The following areas were measured:

a- total area of root dentin;

b- area of dye-infiltrated root dentin.

The percentage of leakage was measured by dividing the dye-infiltrated area by the total area[8].

Statistical Analysis

The qualitative data relative to the scores attributed to the morphological changes were evaluated by the chi-square test. Data relative to the percentage of apical leakage were subjected to Student's t-test for independent samples, by comparing the specimens in the control group with those that were exposed to low-level laser irradiation.

Both tests were conducted using the R statistical software[9], with the significance level at 5%.

RESULT

Morphological Changes

The scores for presence of debris were significantly different ($c^2 = 27$; $p < 0.0001$) between teeth in the control group and those exposed to low-level laser. Specimens in the control group presented higher debris scores. Regarding the presence of open dentinal tubules, significant difference ($c^2 = 60$; $p < 0.0001$) was also observed: teeth that received laser application showed a greater incidence of open dentinal tubules (Figure 1).

As for the occurrence of erosion and cracks, no significant differences were detected between the groups (Figures 2, 3).

Areas of carbonization or other morphological changes were not observed in any specimen, regardless of the group.

Root Dentin Permeability

When the mean ratios of Rhodamine B dye-infiltrated areas divided by the total dentin area were compared, it was verified that the teeth exposed to low-level laser irradiation had significant greater dye leakage in comparison with those in the control group ($t = -6.9987$, $p < 0.001$) (Figure 4).

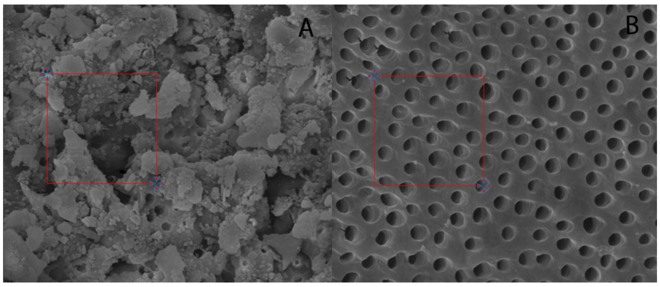

Figure 1. Photomicrograph of the root canal dentin surface of teeth (A) in the control group, showing the presence of debris and few open dentinal tubules; (B) exposed to low-level laser irradiation, showing the absence of debris and presence of open dentinal tubules.

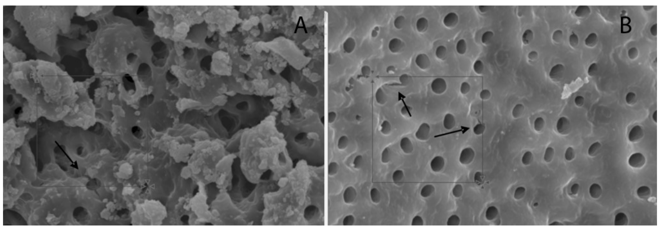

Figure 2. Photomicrograph of the root canal dentin surface (A) in the control group, showing the presence of cracks (arrow); (B) in the group exposed to low-level laser irradiation, showing the presence of cracks (arrow).

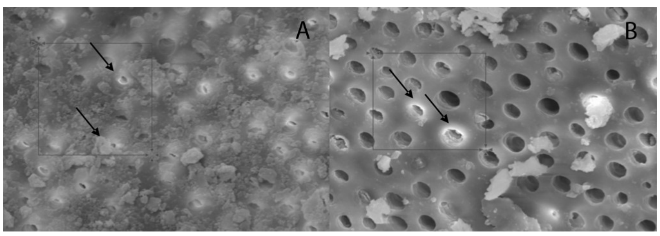

Figure 3. Photomicrograph of the root canal dentin surface (A) in the control group, showing the presence of erosion (arrows); (B) in the group exposed to low-level laser, showing the presence of erosion (arrows).

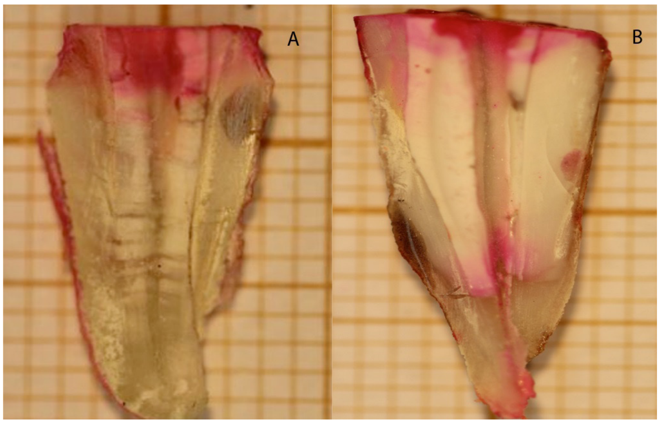

Figure 4. (A) Absence of apical leakage in group 1 (control); (B) Presence of apical leakage in group 2 (teeth that received low-level laser irradiation).

DISCUSSION

Epidemiological data indicate that 30% to 50% of conventional endodontic therapy failures may be attributed to persistent residual infection, which require adjuvant techniques to promote disinfection[10].

Conventional endodontic therapy combined with PDT has shown to be capable of reducing the presence of resistant and biofilm-forming microorganisms within the RCS, as demonstrated by *in vitro, in vivo,* and *ex vivo* studies[11-13]. However, the literature has scarce information regarding morphological changes induced to the dentin structure by PDT.

In the present study, cracks and erosion of dentinal tubules were observed in the teeth exposed to a 660nm laser diode. Nonetheless, these morphological changes were also observed in the group that was not exposed to laser, with the same frequency. Therefore, these morphological changes are likely to be unrelated to the application of 660nm laser.

Furthermore, the absence of carbonized areas of dentin and other morphological changes (such as recrystallization and re-solidification) also constitute an indication that the use of low-level laser does not induce morphological changes in the dentin surface, when used within the parameters described.

We opted to prepare the root canals by means of rotary instrumentation, in order to achieve standardized widening and shaping of the canal, while reducing the time required for completion of CMP. The torque and speed were in accordance to values established by the manufacturers, and were kept constant throughout the instrumentation, for all files. The instrumentation was complemented by irrigation with 5.25% sodium hypochlorite and a final irrigation with 17% EDTA, pH 7.5, aiming to more closely resemble a clinical situation. This technique corroborates previous studies, which indicate the importance of long exposure periods of NaOCl at high concentrations during irrigation, in order to eliminate resistant bacteria[14,15].

Although irrigation using NaOCl followed by EDTA is unable to completely remove the smear layer and debris, this procedure has demonstrated better results than irrigation with each of these solutions separately[5]. Use of EDTA at 17% and pH 7.5 aims to promote further penetration of the solution and remove the smear layer, which occludes dentinal tubules that may contain microorganisms and their sub-products[15].

The results from the present study showed that the smear layer was almost completely removed in the group of teeth subjected to low-level laser irradiation. This group also presented greater incidence of open dentinal tubules. On the other hand, teeth in the control group presented larger amounts of debris on the root canal walls along with occluded dentinal tubules, in spite of the final irrigation with 17% EDTA.

Diffusion of irrigants and intracanal medicaments such as calcium hydroxide and sealers into the dentinal tubules is greater when the smear layer is removed because the presence of debris acts as a physical barrier against these substances by occluding the dentinal tubules[16,17].

This study demonstrated that teeth irradiated with low-level laser presented greater percentages of Rhodamine B dye apical leakage compared with the teeth in the control group, which were not exposed to laser.

The results of the present study indicate that PDT is a promising adjuvant therapy to CMP during endodontic treatment. PDT presented adequate results, without inducing morphological changes to the dentin surface or hindering the permeability of the dentinal tubules in the root dentin. Further *in vivo* and *ex vivo* studies are necessary to determine parameters for the use of low-level laser in daily clinical practice.

CONCLUSION

Photodynamic therapy with 660 nm laser diode combined with chemomechanical preparation of the root canal system resulted in reduction of the smear layer and opening of the dentinal tubules, without inducing morphological changes such as erosion, cracks, or carbonization. In regards to dentin permeability, it was verified that application of laser increased the permeability of the dentinal tubules.

REFERENCES

1. Gomes BP, Pinheiro ET, Sousa EL, Jacinto RC, Zaia AA, Ferraz CC, et al. *Enterococcus faecalis* in dental root canals detected by culture and by polymerase chain reaction analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Aug;102(2):247-53. PMid:16876070. http://dx.doi.org/10.1016/j.tripleo.2005.11.031.
2. Dunavant T, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. J Endod. 2006 Jun;32(6):527-31. PMid:16728243. http://dx.doi.org/10.1016/j.joen.2005.09.001.
3. Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens *ex vivo.* Int Endod J. 2006 Jul;39(7):547-57. PMid:16776759. http://dx.doi.org/10.1111/j.1365-2591.2006.01115.x.

4. Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med. 2004;35(2):111-6. PMid:15334613. http://dx.doi.org/10.1002/lsm.20026.
5. Pécora JD, Brugnera-Junior A, Marchesan MA, Guerisoli DMZ, Barbin EL, Seixas FH. *In vitro* evaluation of external root temperature changes during Er:YAG laser application with five different fiber optic tip withdrawal techniques. Int Congr Ser. 2003 May;1248:297-9. http://dx.doi.org/10.1016/S0531-5131(03)00055-4.
6. Garcez AS, Ribeiro MS, Tegos GP, Nuñez SC, Jorge AO, Hamblin MR. Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection. Lasers Surg Med. 2007 Jan;39(1):59-66. PMid:17066481. http://dx.doi.org/10.1002/lsm.20415.
7. Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, et al. Photodynamic therapy for endodontic disinfection. J Endod. 2006 Oct;32(10):979-84. PMid:16982278. http://dx.doi.org/10.1016/j.joen.2006.04.007.
8. Marchesan MA, Brugnera-Junior A, Souza-Gabriel AE, Correa-Silva SR, Sousa-Neto MD. Ultrastructural analysis of root canal dentine irradiated with 980-nm diode laser energy at different parameters. Photomed Laser Surg. 2008 Jun;26(3):235-40. PMid:18588437. http://dx.doi.org/10.1089/pho.2007.2136.
9. R Core Team. A language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing; 2013 [cited 2013 April 9]. Available from: http://www.R-project.org/
10. Silva LA, Novaes AB Jr, Oliveira RR, Nelson-Filho P, Santamaria M Jr, Silva RA. Antimicrobial photodynamic therapy for the treatment of teeth with apical periodontitis: a histopathological evaluation. J Endod. 2012 Mar;38(3):360-6. PMid:22341075. http://dx.doi.org/10.1016/j.joen.2011.12.023.
11. Fimple JL, Fontana CR, Foschi F, Ruggiero K, Song X, Pagonis TC, et al. Photodynamic treatment of endodontic polymicrobial infection in vitro. J Endod. 2008 Jun;34(6):728-34. PMid:18498901. http://dx.doi.org/10.1016/j.joen.2008.03.011.
12. Garcez AS, Nunez SC, Hamblim MR, Suzuki H, Ribeiro MS. Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. J Endod. 2010 Sep;36(9):1463-6. PMid:20728710. http://dx.doi.org/10.1016/j.joen.2010.06.001.
13. Wilson M. Lethal photosensitization of oral bacteria and its potential application in the photodynamic therapy of oral infections. Photochem Photobiol Sci. 2004 May;3(5):412-8. PMid:15122357. http://dx.doi.org/10.1039/b211266c.
14. Cortez-Retamozo V, Backmann N, Senter PD, Wernery U, De Baetselier P, Muyldermans S, et al. Efficient cancer therapy with a nanobody-based conjugate. Cancer Res. 2004 Apr;64(8):2853-7. PMid:15087403. http://dx.doi.org/10.1158/0008-5472.CAN-03-3935.
15. Souza LC, Brito PR, de Oliveira JC, Alves FR, Moreira EJ, Sampaio HR Fo, et al. Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of Enterococcus faecalis. J Endod. 2010 Feb;36(2):292-6. PMid:20113793. http://dx.doi.org/10.1016/j.joen.2009.09.041.
16. Czonstkowsky M, Wilson EG, Hostein FA. The smear layer in endodontics. Dent Clin North Am. 1990 Jan;34(1):13-25. PMid:2403940.
17. Khedmat S, Shokouhinejad N. Comparison of the efficacy of three chelating agents in smear layer removal. J Endod. 2008 May;34(5):599-602. PMid:18436043. http://dx.doi.org/10.1016/j.joen.2008.02.023.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

*CORRESPONDING AUTHOR

Celso Neiva Campos, UFJF – Universidade Federal de Juiz de Fora, Rua Lindalva de Paula Ribeiro, 240, Bosque Imperial, 36036-466 Juiz de Fora - MG, Brasil, e-mail: cncampos@terra.com.br

Received: May 24, 2016
Accepted: August 24, 2016