In Vivo Aging of Gutta-Percha Dental Cone

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Received 21 July 2005; accepted 20 September 2005
DOI 10.1002/app.23272
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Gutta-percha cone is the most widely used material for root canal filling. The in vivo aging of this cone *focus* on the degradation of its main organic component, *trans*-1,4-polyisoprene, was studied. Aged cones (25 samples) from 2 to 30 years of root canal filling were extracted from different patients in the occasion of retreatment by mechanical way. The information about the aging time was given by the patients. Gel-permeation chromatography (GPC) and infrared spectroscopy (FTIR) were the analytical techniques used. Polyisoprene degrades with time of aging, but in a slow process. Decrease in polymer molar mass from 5.7×10^5 to 1.7×10^5 g/mol was observed in polyisoprene from cone after 30 years of root canal filling and inside a noninfected tooth. In tooth with caries and periodontal infection, the decrease in molar mass is higher (4.6×10^4 g/mol in cone with 10 years of aging). The production of carbonyl and hydroxyl groups in the aged material indicates that the process is oxidative, even in closed teeth. In these cases, the oxygen could be provided from tissue fluid. The degradation mechanism is complex and depends on many factors, besides time of root canal filling. The dental problem caused by the aging could be the production and migration of cytotoxic substances to periodontal ligament and the reduction on the canal sealing property due to the polymer weight loss. Both of them could contribute to the root canal treatment failure. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 4082–4088, 2006

Key words: rubber; dental polymers; biomaterials; biodegradable; ageing

INTRODUCTION

Gutta-percha cone is the most widely used dental material for root canal filling and has been utilized for more than 100 years.[1] The obturation phase of a root filling consist in extraction of pulp and filling the space with a sealer (usually based on ZnO and eugenol) and gutta-percha cone. The success rates of endodontic treatment range between 53 and 94%.[2] However, even though 90% of all endodontic treatment is successful over time, the reciprocal failure is still 10%, which means more than 5 million treatment failure per year, in the United States alone.[2]

Endodontic retreatment is indicated by prosthetic reason or by failure in the first treatment. The prosthetic indication is, in general, motivated by inadequate endodontic obturation. In this case, a coronal leakage or microleakage could occur, promoting the exposition to saliva of obturated canals with subsequent contamination. It is found that root filled canals were completely contaminated after 19 days of coronally contact with bacteria.[3]

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Journal of Applied Polymer Science, Vol. 100, 4082–4088 (2006)

Undoubtedly, the most cause of failure is the infection that remains in the apical portion of canal and/or in the periradicular tissue, even in those cases where the canal has been apparently well treated.[4] The list of organisms most often from endodontic infections is relatively small, compared with normal oral flora, which contains over 500 species of cultivable bacteria. The most frequently identified species from root filled teeth has been *Enterococcus faecalis*, and *Propionibacterium*, *Streptococcus*, and *Lactobacillus* species. *Staphylococcus*, *Eubacterium*, *Actinomyces*, *Fusobacterium*, *Selenomonas*, and *Veillonella* species have also been found.[5]

Trans-1,4-polyisoprene is one of the components of the gutta-percha cones, and represents 14.5–21.8 wt %.[6,7] The others constituents are: wax and resins (1.0–4.1%),[6] ZnO (36.6–84.3%),[7,8] and $BaSO_4$ (0–31.2%).[7,8] Unfortunately, polyisoprenes degrade with time due to aging, and the extent of this degradation depends on many factors, such as temperature, light, chemical environmental (oxygen, ozone, metal),[9,10] biological environmental (microorganisms, enzyme),[11–13] and vinyl unit of rubber.[14] The aging of dental gutta-percha has been studied to verify the effect over mechanical properties[15] and the synergic action of aging and moisture.[16] In these works, however, commercial brands stored in different ambient laboratory conditions (time, temperature, humidity) have been studied to prevent degradation before root canal obturation. No one aging study has been done with the cones in vivo.

In vivo, the cones could be in contact with microbial irritants (e.g., microorganisms, toxins, and metabolites) and products of pulp tissue degeneration that cannot be fully removed during canal cleaning and shaping procedures and that are sealed within the system.[17] Because of sealers dissolution,[18] the cones could also be in contact with tissue fluid and protein through the apical foramen in well-treated root canal or also with bacteria from apical periodontitis. If there is coronal leakage or caries, the oral microbial flora could penetrate the tooth, contaminates the gutta-percha cone, and promotes the degradation of polyisoprene by the large amount of bacteria. Unfortunately, until now, the possibility of gutta-percha degradation inside the root canal is not taken into account by the dentists, even being so probable. The purpose of this work is to study the in vivo aging of gutta-percha cones *focus* on the degradation of *trans*-polyisoprene to understand the influence and importance of such degradation to the root canal filling treatment and durability.

EXPERIMENTAL

Materials

The removal of gutta-percha material from dental canals was done in the occasion of retreatment by mechanical way, with the use of a Hedstroem file (Dentsply-Maillefer, Ballaigues, Switzerland). No solvent was used. The information about the time of root canal filling, considering hereafter as the time of in vivo aging, was given by the patients. Cones from 25 human teeth (20 molars, 3 premolars, 1 incisive, and 1 canine) aged for 2–30 years were extracted from different patients and with mass in the range of 5–20 mg. They were solubilized in chloroform by stirring at ambient temperature (~28°C) overnight. After then, the mixture was filtered in cotton filter to eliminate inorganic or insoluble materials. The solution was centrifuged at 6000 rpm during 10 min, to separate the small solid particles that remain after filtration and obtain clear solutions. Raw gutta-percha was kindly given by Tanariman Industrial Ltda, Amazon, Brazil. It was purified by dissolution in chloroform and precipitation with methanol.

This study was carried out consistent with the principles of the Declaration of Helsinki and with the approval of the Human Research Ethics Committee of the University of Fortaleza, and all patients gave informed consent to participate.

Methods

Gel permeation chromatography

The chromatographic study was performed using a liquid chromatography HPLC system with a refractive index detector, RID-6A, both from Shimadzu (LC-10AD). A series connected system, including a precolumn and two Phenomenex columns, (Linear/Mixed 5 and 5U) was used, employing toluene as the eluent and a flow-rate of 1 mL/min at 25°C. All sample solutions were filtered in PTFE membranes, from Aldrich. The instrument was calibrated with polystyrene standards (Shodex-Showa), with molar mass, M_w, ranged from 1.13×10^3 to 2.15×10^6 g/mol.

FTIR spectroscopic analysis

FTIR spectra of gutta-percha film were registered in Shimadzu model 8300 spectrometer in the range of 4000–400 cm^{-1}. The films were prepared by successive casting and solvent evaporation from polymer solutions in $CHCl_3$ on KBr window. Unaged *trans*-1,4-polyisoprene from purified raw gutta-percha was heated in films at 140°C in air at different time and the thermal oxidation studied by FTIR.

RESULTS AND DISCUSSION

Chromatographic analysis

The chromatograms of the unaged gutta-percha and the extracted material from aged cones are shown in Figure 1. The GPC curve of the unaged gutta-percha is unimodal with peak maximum at 14.5 mL, corresponding to peak molar mass (M_{pk}) of 5.7×10^5 g/mol. A shift of peak maximum to higher elution volume with time of in vivo aging is noted. The consequent decrease in molar mass indicates polymer degradation by polyisoprene backbone cleavage.

The comparison between unaged gutta-percha with that extracted from cone aged for 11, 20, and 30 years, M_{pk} values of 4.5×10^5, 3.6×10^5, and 1.7×10^5 g/mol, suggests a slow process in molar mass decrease with time of in vivo aging. This seems to be the general behavior. A different pattern was observed for the cone aged for 10 years, denoted as 10*, in Figure 1. The elution volume of 17.9 mL with the respective M_{pk} of 4.6×10^4 g/mol indicates a much more intense degradation process.

A much more appropriate overview of the in vivo cone aging process is depicted in Figure 2. It shows the effect of the time of in vivo aging over M_{pk} of extracted gutta-percha. Some points represent average values if more than one sample was available. A major trend of decrease in molar mass with time of in vivo aging was clear. On the other hand, points above and

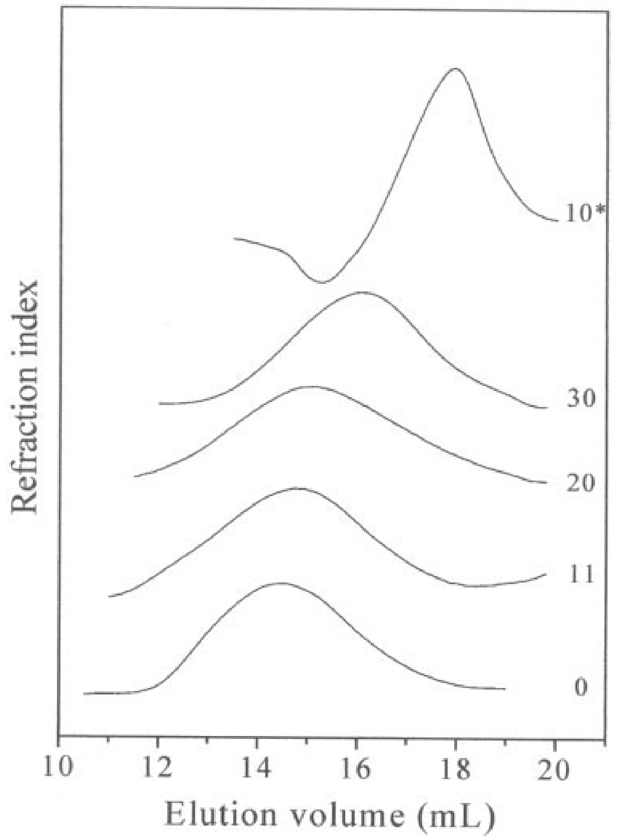

Figure 1 GPC of unaged *trans*-polyisoprene and residual polymer extracted from in vivo aged gutta-percha cone for different times (11, 20, and 30 years and 10 years with infection, denoted as 10*).

below this major curve was observed. The lower M_{pk} values, 4.6×10^4 and 2.5×10^4 g/mol, for 10 and 11 years, respectively, are attributed to an accelerated

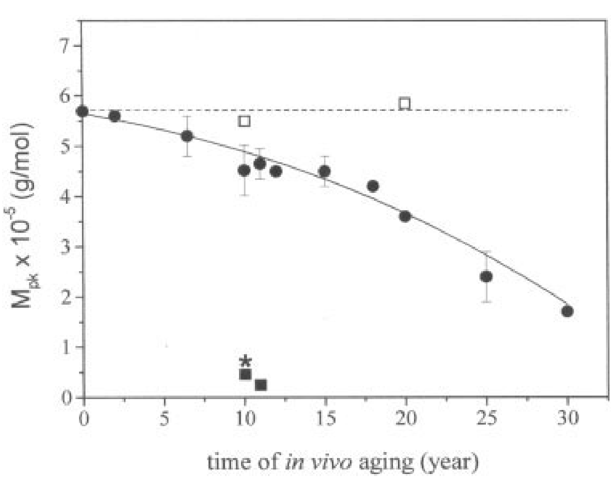

Figure 2 Effect of time of root canal filling over the peak molar mass of residual polyisoprene extracted from in vivo aged gutta-percha cone. Average behavior (●), abnormal behavior: increasing aging (■), apparently unaged sample (□).

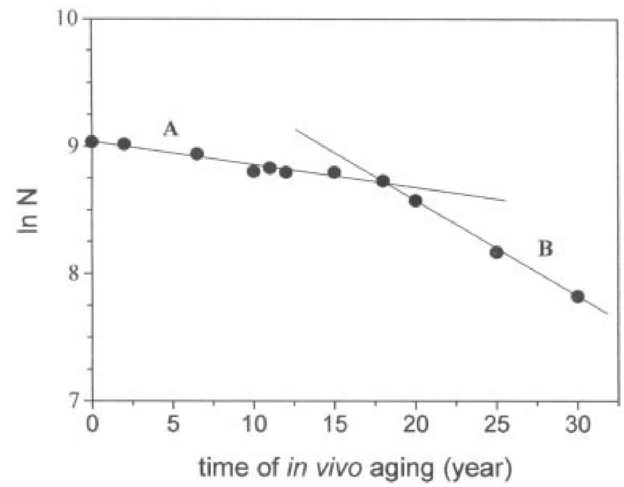

Figure 3 Logarithmic transformation of the number of C═C bonds from polyisoprene extracted during in vivo aging of gutta-percha cone.

aging process. On the contrary, gutta-percha of M_{pk} similar to that of the unaged sample was verified for aged material during 10 and 20 years. A slower degradation could also occur. Points below major curve were associated with a periodontal infection process and caries, detected by radiograph analysis of these teeth done during root canal filling retreatment. Points above curve could indicate preservation in gutta-percha integrity, even after 10 or 20 years of root canal filling treatment. The results registered in Figure 2 confirm the occurrence of polymer degradation, at least in 92% of the analyzed tooth. From these, 84% showed the general behavior depicted in the main curve.

In random degradation, the number of chain cleavages is proportional to the number of linkages N to be broken. This assumption could be represented by the eq. (1), where N_o is the initial number of linkage and k is the apparent rate constant of the degradation and τ_i the induction period.[19] In gutta-percha, the C═C bonds are those involved in chain breaking. By the use of peak molar mass (M_{pk}) values, N could be obtained. The parameter t in the present case is the time of in vivo aging.

$$\ln N = \ln N_o - k(t - \tau_i) \quad (1)$$

Plot of ln N versus time of in vivo aging (Fig. 3) was obtained taking into account the M_{pk} values represented by the main trend. Two straight lines, both of them with good linear correlation coefficient, 0.969 and 0.999, for the first and second lines, respectively, were verified. Line A represents a slower rate aging process that seems to be finished at about 18 years of root canal filling treatment. After then, the degradation becomes faster. The observation of straight lines indicates pseudo first-order processes, as verified for natural rubber thermal oxidation[19] and makes possible the determination of apparent rate constant of aging. Up to 18 years the polymer aging occurs with $k = 1.8 \times 10^{-2}$ year^{-1}. For longer aging, the apparent rate constant increases 4.2 times and the k-value increases to 7.6×10^{-2} year^{-1}. The induction period is close to zero, which means that the degradation, even slow, initiates as soon as the root canal is filled or after few months.

Spectroscopic study

Degradation process includes structural changes that can be analyzed by FTIR. Figure 4 shows the spectra of some representative extracted gutta-percha material in comparison with the unaged gutta-percha spectrum. The band assignments are depicted in Table I. The spectrum of unaged *trans*-1,4-polyisoprene shows characteristics bands of the polymer.[21] A nonexpected absorption at 1737 cm^{-1} was present in this material, and indicates that the analyzed gutta-percha polymer has a small degree of oxidation and/or contains small amount of protein. Three important regions could be considered after aging: (a) 3400–3420 cm^{-1} attributed to OH stretching; (b) 1715–1737 cm^{-1}, due to C═O stretching; and (c) 797–881 cm^{-1}, attributed to ═C—H bending from *trans*-1,4 isomer. All aged cones showed the presence of OH and C═O groups. They probably correspond to product of degradation, such as alcohols, carboxylic acid, hydroperoxide, aldehyde, ketone, ether or ester, some of them present in oxidation of polyisoprenes.[23] The important point is that the aging process includes oxidation, even in apparently well-treated teeth in which there is no coronal leakage or caries that make possible the contact between the gutta-percha and the atmospheric oxygen from the mouth. The origin of the oxygen could be the tissue fluid that contains 40 mmHg of this element and permeates all body tissues.[24]

The pattern of region (c) in aged material is different from that of unaged gutta-percha. The disappearance of the bands at 880, 860, and 800 cm^{-1}, characteristics of crystalline form, and the remaining of the band at 835–840 cm^{-1} indicates that during the aging the *trans*-1,4-polyisoprene becomes amorphous.[22] The formation of oxygenated products, crosslinking, and cleavage of the main chain may be responsible for reducing crystallinity.

The polyisoprene extracted from cone aged for 10 years and from an infected root canal, denoted as 10*, presents similar spectrum of the other aged samples, but with higher absorbance of OH and C═O bands. Two other differences become clear: increase in band absorbance at 1078–1099 and 1028–1039 cm^{-1} and decrease at 835–840 cm^{-1}. The first one is attributed to C—O stretching from ester group, formed during the degradation. The second difference is the decrease in the relative amount of ═C—H bending, caused by the diminishing amount of C═C bonds. This could be associated with the chain cleavage and the decrease in molar mass from 5.7×10^5 to 4.6×10^4 g/mol, as observed in chromatographic study.

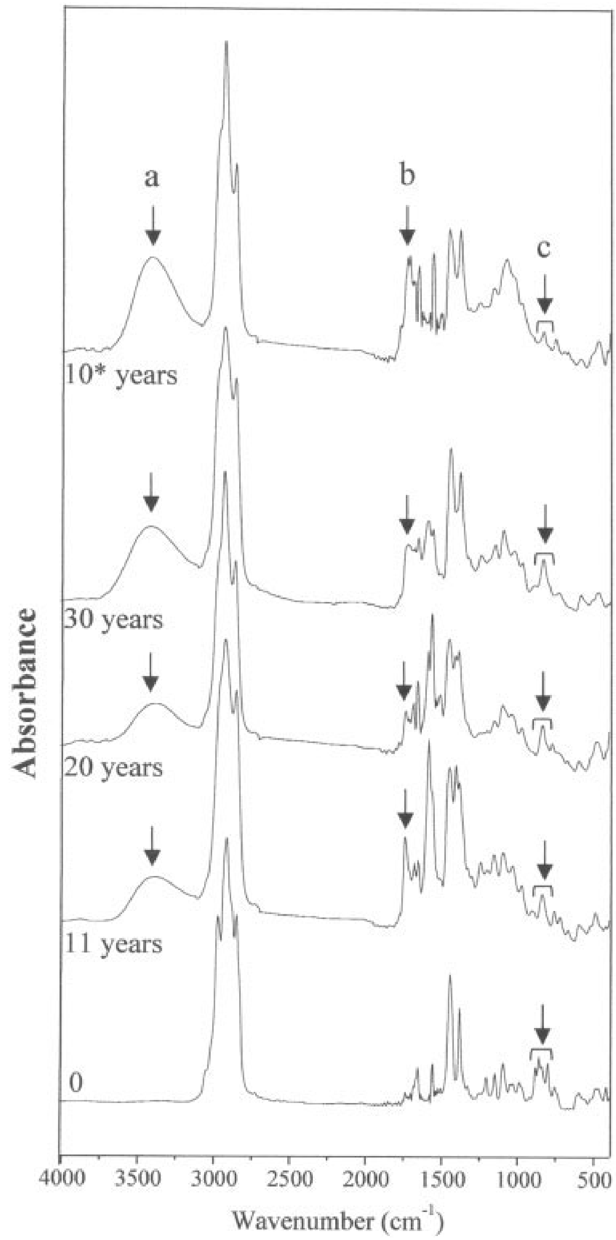

Figure 4 FTIR spectra of unaged *trans*-polyisoprene and polymer extracted from in vivo aged gutta-percha cone for different times (11, 20, and 30 years and 10 years with infection, denoted as 10*).

In thermal oxidation of *trans*-1,4-polyisoprene films at 140°C an increase in C═O stretching (1720 cm^{-1}) and OH stretching (3448 cm^{-1}) absorbance was observed, as well as a decrease in ═C—H bending (835 cm^{-1}) (data not shown). There is also an increase in the region around 1100 cm^{-1}. The same spectral

TABLE I
FTIR Bands Assignments of Unaged Gutta-Percha and Residual Polymer Extracted from Aged Cones

Wavenumber (cm^{-1})					
Unaged	Aged	Literature	Assignment	Origin	Ref
–	3400–3420	3300–3400	ν of O—H	Hydroperoxides, alcohol, carboxylic acid	20
3052	3045	3052	ν of =C—H	*trans*-isoprene	21
2964	2959–2960	2961	ν_{as} of CH_3	*trans*-isoprene	21
2916–2918	2918–2924	2930, 2910	ν_{as} of CH_2	*trans*-isoprene	21
2851	2852–2854	2850	ν_s of CH_2	*trans*-isoprene	21
–	1715–1737	1710–1750	ν of C=O	Aldehyde, ketone, carboxylic acid, ester	20
–	1678–1683	1675–1681	ν of C=C	C=C in a ring or ketone	20
		1680	ν of C=O	C=O in resonance with C=C	
1657–1666	1653–1657	1667	ν of C=C	*trans*-isoprene	21
1445	1445–1452	1445	δ of CH_2	*trans*-isoprene	21
1381	1381–1383	1384	δ_{as} of CH_3	*trans*-isoprene	21
1205	1205–1228	1210	β of =C—H	*trans*-isoprene	21
1150	1151–1160	1154	ω of CH_3	*trans*-isoprene	21
1096		1101	ν of C—C	*trans*-isoprene	21
	1078–1099		ν of C—O	Ester	20
	1028–1039		ν of C—O	Ester	20
1030		1035	ρ of CH_3	*trans*-isoprene	21
990	972–976	990	ν of C—C	*trans*-isoprene	21
881	875–881	884	γ of =C—H	*trans*-isoprene	21
860	862	858	γ of =C—H	*trans*-isoprene	22
843	835–840	843	γ of =C—H	*trans*-isoprene	22
800	797–800	815	γ of =C—H	*trans*-isoprene	22
756	748–760	769	ρ of CH_2	*trans*-isoprene	23

ν, stretching; δ, deformation; β, in-plane bending; γ, out of plane bending; ω, wagging; ρ, rocking; *s*, symmetric; as, asymmetric.

changes are observed in vivo aging of gutta-percha cone (Fig. 4). The comparison of the in vivo degradation with the in vitro (thermal oxidation) could also be seen in Figure 5. The relative absorbance at 3400 cm^{-1} versus time of heating (in vitro aging) [Fig. 5(A)] follows a sigmoidal dependence, as verified for thermal oxidation of polyisoprene.[25] Different behavior of in vivo degradation was observed [Fig. 5(B)]. In this plot, there is no clear dependence of OH group formed during the degradation, and the time scale is higher. The large dispersion of point is characteristic of complex and many variables dependent process, as considered before.

In vivo degradation variables

The complexity of the in vivo aging could be related with many factors, the first one is about the agent of degradation, whether it includes microbes or not. Infection process makes possible microbial degradation, considered as effective way of polyisoprene degradation.[11–13] Many microorganisms have been reported to

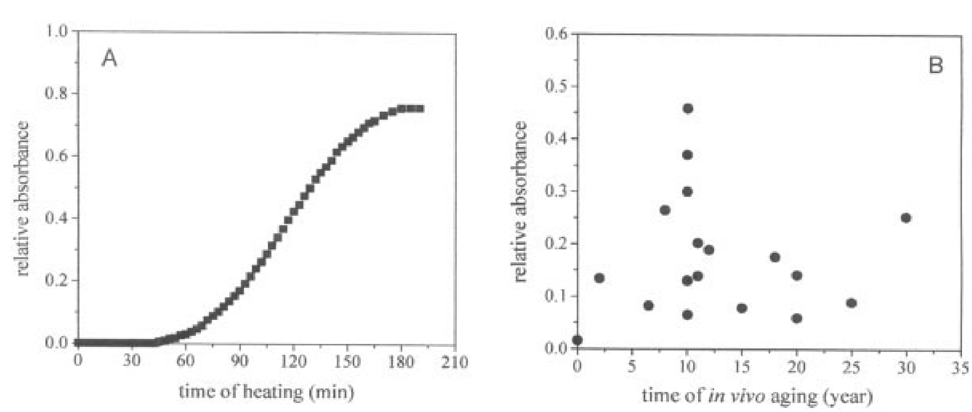

Figure 5 Effect of the time of gutta-percha cone aging over the amount of hydroxyl groups formed during degradation: (A) thermal aging of gutta-percha polymer at 140°C in oxygen atmosphere; (B) in vivo aging.

degrade polyisoprene rubbers. The most common belong to actinomycetes, such as *Streptomyces*, *Amycolatopsis*, and *Nocardia* sp.[12] Actinomycetes is one of the bacteria found in root canals infection of teeth[5] that could promote *trans*-1,4-polyisoprene degradation. The biodegradation of the polyisoprene is poorly understood,[11] but it is assumed to occur via oxidative cleavage of double bonds,[11–13] as was verified in the in vivo aging of gutta-percha cone. The microbial degradation of gutta-percha cones is under investigation in our research group now.

Many other factors could influence the in vivo aging of gutta-percha, such as those related to: (a) teeth anatomy, (b) the root canal treatment, and (c) cone characteristics. The most important point of teeth anatomy could be the apical foramen dimension or obliteration. The apical foramen is the orifice that communicates the inside part of the root canal with the periodontal ligament (outside the tooth, close to the bone). It is through this orifice that the tissue fluid and the oxygen from it could get in contact with the polymer. Obliteration avoids the contact and may provide less degree of degradation.

During the root canal filling treatment, the cone heating temperature used in the treatment could be the most significant variable. In the failure treatment, with caries or leakage, the contact between cone and saliva and so the composition of saliva could also be determinable factors. The effect of the main cone components, such as ZnO, $BaSO_4$, or wax/resin over the degradation of *trans*-polyisoprene is unknown. On the other hand, some minor components, such as those that contain transition metal ions, for example, iron used as pigments, is well known as pro-oxidant for polyisoprene and certainly play an important role on the aging process. The effect of some of these components is being studied at the moment by the authors.

Possible dental problems caused by gutta-percha degradation

The first possible dental problem caused by the gutta-percha degradation is the release through apical foramen to periodontal ligament, of low-molecular weight substances that could cause cytotoxicity. It is known that levunaldehyde, methacrolein, methyl vinyl ketone, 4-hydroxy-2-butanone, 4-hydroxy-4-methyl-5-hexene-al, 4-methyl-4-vinyl-butyrolactone, and 5-hydroxy-6-methyl-6-heptene-2-one are produced during the *cis*-polyisoprene thermal-oxidation.[26] Low-molecular weight oligo(*cis*-1,4-isoprene) molecules with aldehyde and keto groups at their respective ends have also been identified as products of *cis*-1,4-polyisoprene bacterial degradation.[11] Isoprene fragments were liberated from enzymatic and nonenzymatic lipid peroxidation of polyisoprene vulcanized rubber.[12] Isoprene itself have caused cytotoxicity and interleukin-8 (IL-8) gene expressions, as a market for inflammation, and its photochemical degradation products, such as methacrolein and methyl vinyl ketone, significantly enhanced cytotoxicity and IL-8 gene expression and induce potentially greater adverse health effect.[27]

The second possible problem could be the polymer weight loss. The *cis*-1,4-polyisoprene degraded by bacteria, for example, showed weight losses up to 18% for 10 weeks of incubation at 30°C.[11] Still more weight loss (80%) was verified in the oxidation of vulcanized rubbers in lipid peroxidation initiated by Fenton reaction (reaction between Fe(II) and H_2O_2) at 30°C.[12] The weight loss in gutta-percha polymer could make the cone more porous and reduce its root canal sealing property, essential for the success and durability of this dental treatment.

CONCLUSIONS

Trans-1,4-polyisoprene from gutta-percha cone degrades with time of root canal filling. In the average behavior, represented by 84% of the analyzed teeth, there is no induction period, which means that the aging process begins as soon as the teeth are treated and the gutta-percha cones are sealed inside the root canal or after few months. However, the degradation occurs slowly. Up to 18 years of aging, the degradation apparent rate constant is 1.8×10^{-2} year^{-1}. For longer time, the degradation becomes four times faster.

Two main changes were verified during in vivo aging: (a) the decrease in polymer molar mass, as an indicative of polymer backbone cleavage, and (b) the production of carbonyl and hydroxyl groups in the residual polymer. This indicates that the aging process involves reaction with oxygen and explains the low rate constant, probably caused by the low available oxygen content inside the root canal. High abnormal degree of aging was observed in infected teeth, and suggests the participation of microorganisms in the polymer degradation.

The degradation mechanism is complex and seems to be influenced by many variables, besides time of aging. Some possible variables could be apical foramen dimension or obliteration, amount and kind of bacteria from an infection, amount of available oxygen, contact between cone and saliva, saliva composition, cone composition, and cone heating temperature used in the root canal treatment. The effect of bacteria and cone components is under investigation now.

The gutta-percha aging could be an important factor on the root canal treatment durability, principally because of possible migration of cytotoxic degradation products to periodontal tissue and reduction on sealing property caused by polymer weight loss.

The authors thank Tanariman Industrial Ltda for supplying the gutta-percha. This work was supported by CNPq, CAPES, and FUNCAP.

References

1. Vajrabhaya, L.-O.; Suwannawong, S. K.; Kamolroongwarakul, R.; Pewklieng, L. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004, 98, 756.
2. Ruddle, C. J. In Pathways of the Pulp, 8th ed.; Cohen, S., Burns, R. C., Eds.; Mosby: St. Louis, MO, 2002; p 875.
3. Torabinejad, M.; Borasmyt, U.; Kettering, J. D. J Endod 1990, 16, 556.
4. Lopes, H. P.; Siqueira, J. F., Jr. Endodontia, Biologia e Técnica; Editora Médica e Científica Ltda: Rio de Janeiro, 1999; p 497.
5. Adib, V.; Spratt, D.; Ng, Y.-L.; Gulabivala, K. Int Endod J 2004, 37, 542.
6. Friedman, C. E.; Sandrick, J. L.; Heuer, M. A.; Rapp, G. W. J Endod 1977, 3, 304.
7. Gurgel-Filho, E. D.; Feitosa, J. P. A.; Teixeira, F. B.; de Paula, R. C. M.; Silva, J. B. A., Jr; Souza-Filho, F. J. Int Endod J 2003, 36, 302.
8. Marciano, J.; Michailesco, P. M. J Endod 1989, 15, 149.
9. Li, G.-Y.; Koenig, J. L. Polym Degrad Stab 2003, 81, 377.
10. Rodrigues, F. H. A.; Feitosa, J. P. A.; Ricardo, N. M. P. S.; Heathey, F. Polym Int 2004, 53, 733.
11. Bode, H. B.; Kerkhoff, K.; Jendrossek, D. Biomacromolecules 2001, 2, 295.
12. Sato, S.; Honda, Y.; Kuwahara, M.; Watanabe, T. Biomacromolecules 2003, 4, 321.
13. Enoki, M.; Dol, Y.; Iwata, T. Biomacromolecules 2003, 4, 314.
14. Ivan, G.; Meghea, A.; Giurginca, M.; Iftimie, N. Polym Degrad Stab 2003, 80, 397.
15. Oliet, S.; Sorin, S. M. Oral Surg Oral Med Oral Pathol 1977, 43, 955.
16. Arvanitoyannis, I.; Kolokuris, I.; Robinson, C.; Blanshard, J. M. V. J Appl Polym Sci 1993, 47, 1905.
17. Gutmann, J. L.; Witherspoonn, D. E. In Pathways of the Pulp, 8th ed.; Cohen, S., Burns, R. C., Eds.; Mosby: St. Louis, MO, 2002; p 295.
18. Kazemi, R. B.; Safavi, K. E.; Spangberg, L. S. W. Oral Surg Oral Med Oral Pathol 1993, 76, 766.
19. Rodrigues, J. F.; Bezerra, C. C. Polym Bull 1996, 36, 347.
20. Pavia, D. L.; Lampman, G. M.; Kriz, G. S. Introduction to Spectroscopy: A Guide for Students of Organic Chemistry, 2nd ed.; Saunders College Publishing: Florida, 1996; Chapter 2.
21. Arjunan, V.; Subramanian, S.; Mohan, S. Spectrochim Acta Part A 2001, 57, 2547.
22. Richardson, W. S.; Sacher, A. J Polym Sci 1953, 10, 353.
23. Alam, T. M.; Celina, M.; Assink, R. A.; Clough, R. L.; Gillen, K. T.; Wheeler, D. R. Macromolecules 2000, 33, 1181.
24. Guyton, A. C. Textbook of Medical Physiology, 8th ed.; Saunders: Philadelphia, 1991.
25. Ricardo, N. M. P. S.; Feitosa, J. P. A.; Silveira, E. R.; Almeida Neto, M. P. Polym Bull 2001, 46, 107.
26. Barnard, D.; Lewis, P. M. In Natural Rubber Science and Technology; Roberts, A. D., Ed.; Oxford University Press: Oxford, 1988; p 621.
27. Doyle, M.; Sexton, K. G.; Jeffries, D.; Bridge, K.; Jaspers, I. Environ Health Perspect 2004, 112, 1488.