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Degradation of *trans*-polyisoprene over time following the analysis of root fillings removed during conventional retreatment

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Abstract

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Aim To evaluate *in vivo* degradation of root filling materials over time.

Methodology Thirty-six root filled teeth with or without periapical lesions were selected. Teeth with poor coronal restoration were not included. The teeth had been root filled 3–30 years previous and were scheduled for conventional retreatment. The association of root canal treatment, age, periapical lesion and root filling degradation was investigated. The filling material was removed from the root canal using files and no solvent. *Trans*-1,4-polyisoprene was isolated through solubilization of root filling remnants in chloroform followed by filtration and centrifugation. Gel permeation chromatography (GPC) and infrared spectroscopy (FT-IR) were utilized to study the occurrence and degree of degradation. The GPC and FT-IR data were collected for each sample and analysed statistically using the Kruskal–Wallis test.

Results Degradation of *trans*-1,4-polyisoprene was a slow process. The process was identified as an oxidation reaction through the production of carboxyl and hydroxyl groups. Compared with the control group, significant molar mass decrease was noted after 15 years ($P = 0.0146$) in teeth with no periapical lesions. However, in teeth associated with periapical lesions the number of years for significant degradation was reduced to 5 ($P = 0.0009$).

Conclusion Polyisoprene degrades inside root canals as an oxidative process. The presence of periapical lesions was associated with a more rapid onset of degradation.

Keywords: *in vivo* degradation, root canal therapy, *trans*-polyisoprene, treatment outcome.

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Introduction

Re-infection of the root canal system is one of the factors that influences the outcome of root canal treatment (Ray & Trope 1995, Zaia *et al.* 2002). Bacteria, or their products, are considered to be the primary aetiological agents of periradicular pathosis (Kakehashi *et al.* 1965, Takahashi 1998). Therefore, their elimination is one of the most important steps in root canal treatment (Pinheiro *et al.* 2003). When the root canal is only partially cleaned, shaped and filled the association with disease increases (Sjögren *et al.* 1997). Persisting bacteria in root canals have survived biomechanical procedures, and are often located in non-instrumented areas (Fukushima *et al.* 1990). The root filling aims at entombing bacteria and also prevents re-infection from a coronal direction (Pinheiro *et al.* 2003).

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The purpose of the filling phase of root canal treatment is to leave the tooth in the most biologically inert condition possible (Carrotte 2004) by preventing microorganisms from re-entering the root canal system, and isolating any microorganisms that may remain within the tooth from nutrients in tissue fluids (Vivacqua-Gomes *et al.* 2005). It is generally believed that the amount of sealer should be minimized in favour of gutta-percha. This supposition is based on reports that sealers dissolve over time and shrink during setting, leaving voids (Georgopoulou *et al.* 1995). In fact, dissolution of zinc eugenol-oxide endodontic sealers was shown to start within 3 h of exposure to the apical region and to continue for at least 6 months (Kazemi *et al.* 1993).

Retreatment of an existing root filling is indicated for prosthetic reasons or because of post-treatment disease (Sjögren *et al.* 1997, Pinheiro *et al.* 2003).

Gutta-percha is the most widely used material for root fillings and has been used for more than 100 years (Tagger & Gold 1988, Vajrabhaya *et al.* 2004). Polyisoprene (*trans*-1,4) is one of the components of gutta-percha cones, and represents 14.5–21.8% of its weight (Friedman *et al.* 1977, Gurgel-Filho *et al.* 2003). The other constituents are: wax, resins (1.0–10.4%) (Friedman *et al.* 1977), ZnO (36.6–84.3%) and $BaSO_4$ (0–31.2%) (Marciano & Michailesco 1989, Gurgel-Filho *et al.* 2003, Maniglia-Ferreira *et al.* 2005). Unfortunately, degradation of polyisoprene can occur as a result of several factors such as increase in temperature, exposure to light, as well as to chemical and biological environmental changes (Sawada 1986, Somers *et al.* 2000, Bode *et al.* 2001, Enoki *et al.* 2003, Sato *et al.* 2003).

The ageing of dental gutta-percha has been studied in order to verify the effect of time on its mechanical properties (Oliet & Sorin 1977), and the synergic action of age and moisture on the material (Arvanitoyannis *et al.* 1993). Arvanitoyannis *et al.* (1993) studied commercial brands of gutta-percha stored in different laboratory environment conditions (time, temperature, humidity) to prevent degradation before root canal filling. Recently, chemical aspects of *in vivo* ageing of gutta-percha cones have been reported (Silva *et al.* 2006). However, the consequence of this degradation of gutta-percha on the long-term outcome of root canal treatment was not discussed.

The purpose of this work was to determine the degradation of a root filling material (*trans*-polyisoprene), removed from root canals that had been placed for periods of between 3 and 30 years.

Materials and methods

Samples

Thirty-six patients with teeth with or without periapical lesions and who required non-surgical root canal retreatment for prosthetic reasons were selected from those attending the Fortaleza Dental School (University of Fortaleza), CE, Brazil. A detailed medical and dental history was obtained from each patient. The Ethical Committee in Research of the Dental School of Fortaleza (University of Fortaleza) approved a protocol describing the sample collection for this investigation. Teeth with poor coronal restorations (any permanent restoration with radiographic signs of overhangs, recurrent decay or open margins) were excluded (Tronstad *et al.* 2000). The filling material within 36 roots, treated 3–30 years previous, were removed and analysed. The patients provided information on the date when the root canal fillings were performed.

Sampling procedures

After access cavity preparation, the teeth were individually isolated from the oral cavity with a rubber dam and disinfected with a 2% chlorhexidine solution. The removal of filling materials from the root canals was completed using Hedströem files (Dentsply Maillefer, Ballaigues, Switzerland) without solvent. The samples were than submitted to dissolution in chloroform (Synth; Diadema, São Paulo, Brazil) over night by stirring at room temperature (28 °C). After this, the mixture was passed through a cotton filter to remove inorganic or insoluble materials. The solution was centrifuged (Andreas Hettich GmbH & Co., KG, Tuttlingen, Germany) at 6000 rpm for 10 min to separate the small solid particles that remained after filtration and to obtain clear solutions. Gel permeation chromatography (GPC) and infrared spectroscopy (FT-IR) were then used to analyse the materials. Raw gutta-percha (Tanariman Industrial Ltd, Manaus, Amazon, Brazil) used for the production of gutta-percha cones was purified by dissolution in chloroform and precipitation with methanol (Synth, Diadema). This gutta-percha was included as a control in relation to the degradation because it was a non-manipulated polymer, recently obtained and had not undergone degradation.

Three different cases from each chosen year were collected (12 different periods) for the analyses of the filing material, resulting in 36 samples. Their periapical conditions were also considered . The final values for GPC and FT-IR were taken as the averages of the results obtained for all the specimens of every age and periapical condition. Therefore triplicate samples were obtained that had similar data, indicating coherence in our results.

Gel permeation chromatography

The chromatographic study was performed using a high-performance liquid chromatography system with a refractive index detector, RID-6A (LC-10AD; Shimadzu, Tokyo, Japan). A series connected system including a pre-column and two Phenomenex columns (Linear/Mixed 5 and 5U) was used, and toluene (Synth, Diadema) was employed as the eluent at a flow rate of 1 mL min^{-1} at 25 °C. All sample solutions were filtrated in PTFE membranes (Sigma-Aldrich Co., St Louis, MO, USA). The instrument was calibrated with polystyrene standards (Shodex-Showa, Orlando, FL, USA), with molar mass, M_w, ranging from 1.13×10^3 to 2.15×10^6 g mol^{-1}.

Gel permeation chromatography is a method used for the determination of the molecular weight of polymers. It employs a liquid chromatography that separates molecules according to molecular weight (Elias 1997).

FT-IR spectroscopy analysis

FT-IR spectra of gutta-percha film were registered in a 8300 spectrometer model (Shimadzu) in the range of 4000 to 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 into films at 140 °C in air at different periods and thermal oxidations. The samples were then studied by FT-IR spectroscopy.

Chemical groups were identified by FT-IR spectroscopy, which is an analysis based on the vibrational and rotational motions of a molecular bond (Pavia *et al.* 1996).

Statistical analysis

The data collected for each sample were entered into a spreadsheet and analysed statistically using SPSS for Windows (SPSS Inc., Chicago, IL, USA). The Kruskal–Wallis test was used to test the null hypothesis that there is no relationship between the root filling material, ageing and degradation of *trans*-1,4-polyisoprene.

Results

Data obtained from FT-IR spectrum (Fig. 1) and GPC (peak molar mass and elution volume) for the root filling samples are shown in Table 1. The GPC curve of the unaged gutta-percha was unimodal with a maximum peak at 14.5 mL, corresponding to a molar mass peak (M_{pk}) of 5.7×10^5 g mol^{-1}, in agreement with the values reported, ranging from 1×10^5 to 2.5×10^6 g mol^{-1}. A maximum peak shift to higher elution volume for root canals filled at different ages was noted. The consequent decrease in molar mass indicated polymer degradation by the backbone cleavage of polyisoprene (Table 1).

After 15 years the root filling materials demonstrated a decrease of C=C bonds, and an increase of OH and C=O formation, confirming degradation of polyisoprene

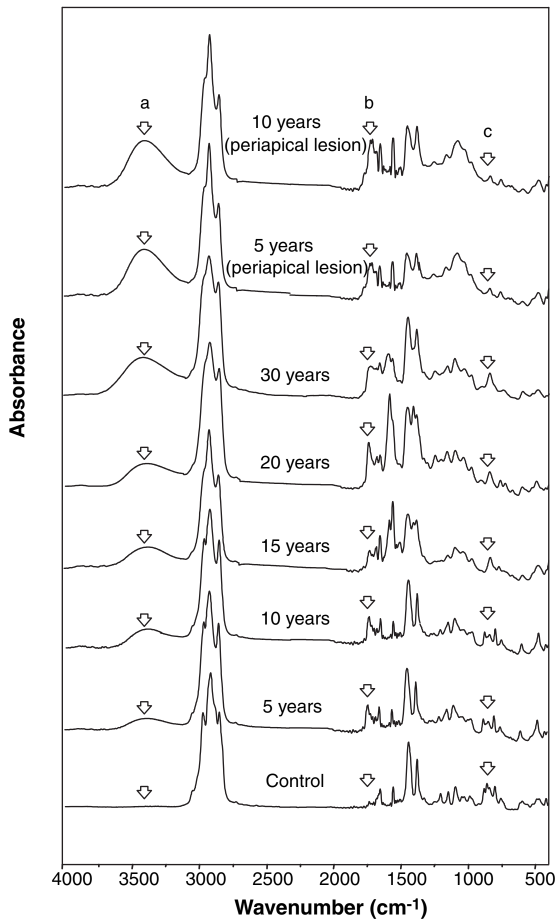

Figure 1 FT-IR spectra of non-aged *trans*-polyisoprene and polymer extracted from gutta-percha cone aged *in vivo* for different periods (0, 5, 10, 15, 20, and 30 years without periapical lesion and 5 and 10 years with periapical lesion).

Table 1 FT-IR and GPC results obtained from polyisoprene extracted from filling materials

	GPC		Estimated ageing degree from FT-IR		
Ageing time (years)	Elution volume (mL)	$^{a}M_{pk} \times 10^{-5}$ (g mol^{-1})	OH formation	C=O formation	Average value
Control	14.5	5.7	1	5	3.0
5	14.6	5.2	7	13	10.0
10	14.8	4.5	18	15	16.5
15	16.1	3.8	30	25	27.5
20	17.0	3.6	33	28	30.5
30	18.7	1.7	50	33	41.5
5[b]	17.8	2.6	36	31	33.5
10[b]	21.7	0.46	79	62	70.5

[a]M_{pk} is peak molar mass of polyisoprene.
[b]Presence of periapical lesion (root canal infection).

and formation of volatile products, suggesting that the polymer lost weight during degradation. Degradation of *trans*-1,4-polyisoprene was a slow process. The process is identified as an oxidation reaction through the production of carboxyl and hydroxyl groups. Compared with the control group, significant molar mass decrease was noted after 15 years ($P = 0.0146$) in teeth with no periapical lesions. However, in teeth associated with periapical lesions the number of years for significant degradation was reduced to 5 ($P = 0.0009$).

Discussion

The degradation of *trans*-1,4-polyisoprene occurs as a result of temperature, exposure to light, as well as to chemical (oxygen, ozone, metal) (Somers *et al.* 2000, Rodrigues *et al.* 2004) and biological environmental shifts (microorganisms, enzyme) (Bode *et al.* 2001, Enoki *et al.* 2003, Sato *et al.* 2003).

The degree of degradation increased with time of ageing (Table 1). Comparison between degradation intensity for filling material aged for 5 and 10 years in teeth without apical pathosis and those with apical pathosis revealed that ageing depended on other factors besides time. No induction period was observed, indicating that the degradation process begins immediately after gutta-percha cones are placed inside the root canal, or in fact, after they are produced. However, degradation is a slow process.

The degradation reaction includes chemical changes that can be analysed by FT-IR. Three important regions, using the FT-IR spectra (Fig. 1) could be considered after ageing: (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 demonstrated the presence of OH and C=O groups. They correspond to degradation products such as alcohols, carboxylic acid, hydroperoxide, aldehyde, ketone, ether or ester, of which some are present in the oxidation of polyisoprenes (Alam *et al.* 2000). The presence of groups containing oxygen indicates that the ageing process includes oxidation, as reported by Silva *et al.* (2006).

According to Silva *et al.* (2006), two main changes were verified during *in vivo* ageing: (i) the decrease in polymer molar mass, as an indication of polymer backbone cleavage; and (ii) the production of carboxyl (C=O) and hydroxyl (OH) groups from the residual polymer. This indicates that the ageing process involves a reaction with oxygen, which explains the low constant rate, probably caused by the low oxygen content available inside the root canal. An abnormally high degree of ageing was observed in teeth with apical pathosis (Fig. 1; 5 and 10 years), which may be related to the presence of microorganisms and a possible raise in polymer degradation, as proposed by Silva *et al.* (2006).

The results showed that 15 years after root canal treatment, the composition of gutta-percha can be modified substantially with a resultant loss in the mass of the root canal filling material, that may result in empty spaces inside the root canal system that could permit bacterial re-colonization. Thus, the degradation process may be associated with post-treatment disease and the outcome of root canal treatment (Sjögren *et al.* 1997, Pinheiro *et al.* 2003, Valois *et al.* 2005).

As expected, a decrease of the molar mass was associated with the increase of age. This fact can be attributed to depolymerization (Somers *et al.* 2000). Cones in teeth for 10 years with apical pathosis, revealed the greatest degree of ageing (71%), in agreement with results shown in Fig. 1 (10 periapical lesions) and with results found by Silva *et al.* (2006).

Taking into account that the *in vivo* ageing process is an oxidation reaction and could be associated with microbial degradation, some variables can be important, such as the presence of voids in root canal fillings, apical foramen dimension or obliteration, amount and kind of bacteria from infection, amount of oxygen available and coronal and apical leakage. The degradation mechanism is complex and seems to be influenced by these many variables.

Many microorganisms have been reported to degrade polyisoprene rubbers. The most common belong to actinomyces species such as Streptomyces, Amycolatopsis and Nocardia sp. (Sato *et al.* 2003). Actinomyces is one of the bacteria found in root canal infection (Adib *et al.* 2004) that could promote *trans*-1,4-polyisoprene degradation. The biochemical and molecular basis of polyisoprene degradation is poorly understood (Bode *et al.* 2001). It is assumed that biodegradation of the polymer backbone occurred via oxidative cleavage of double bonds (Bode *et al.* 2001, Enoki *et al.* 2003, Sato *et al.* 2003), as was verified in the *in vivo* ageing of gutta-percha cones.

Another important effect of volatile product formation during degradation is the weight loss of the polymer. Determination of residual weight of the *cis*-1,4-polyisoprene sample degraded by bacteria, for example, showed weight losses of up to 18% after 10 weeks of incubation at 30 °C (Bode *et al.* 2001). A greater weight loss (80%) was also 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 (Sato *et al.* 2003). The weight loss of the gutta-percha polymer could make the material more porous and reduce its root canal sealing property.

Gutta-percha ageing could be an important factor for root canal filling longevity, principally because of possible migration of cytotoxic degradation products to periodontal tissue and reduction of the sealing property caused by polymer weight loss (Silva *et al.* 2006).

Conclusion

Polyisoprene degrades inside the root canal. The degradation begins soon after the root treatment, but occurs slowly up to 15 years. The process is oxidative and its intensity seems to depend on factors besides time of ageing. An increase in degradation was noted in teeth with apical pathosis.

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