Clinical Relevance of *Trans* 1,4-Polyisoprene Aging Degradation on the Longevity of Root Canal Treatment

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This *in vivo* study investigated the time of degradation of root filling material (*trans* 1,4-polyisoprene) retrieved from endodontically treated teeth and correlated the occurrence of degradation with the longevity of endodontics. Thirty-six root-filled teeth with different filling times (2 to 30 years) and with and without periapical lesions were selected. All teeth presented clinical indication for root canal retreatment. The association among filling time, presence of periapical lesion and root filling material degradation was investigated. Root filling samples were retrieved from the root canals using a Hedström file without solvent. The *trans* 1,4-polyisoprene was isolated by root filling solubilization in chloroform followed by filtration and centrifugation. GPC and FT-IR were the analytical techniques utilized. Degradation of *trans* 1,4-polyisoprene occurred with time, as a slow process. It is an oxidative process, and production of carboxyl and hydroxyl groups in the residual polymer were observed. Statistically significant decrease of molar mass was observed after 5 (p=0.0001) and 15 (p=0.01) years in teeth with and without periapical lesion, respectively. Bacteria participated in polymer degradation. Gutta-percha aging was proven an important factor for the long-term success of endodontic treatment. The findings of the present study showed that, after 15 years, polymer weight loss may decrease the capacity of the filling mass to seal the root canal space and prevent re-infection, thus compromising significantly the longevity of root canal therapy.

Key Words: root canal therapy, treatment outcome, *trans* 1,4-polyisoprene, *in vivo* degradation.

INTRODUCTION

The main goal of root canal treatment is to prevent or heal apical periodontitis by cleaning, shaping and filing the root canal space. The root canal system has a complex anatomy, characterized by the presence of curvatures, accessory and lateral canals, deltas and isthmuses (1). Ideally, endodontics should provide three-dimensional filling of the main root canal and accessory canals (2).

Re-infection of the root canal system is one of the key factors that influence the treatment outcome (3). Bacteria and their byproducts are considered to be the primary etiological agents of apical periodontitis (3,4). Therefore, their elimination is one of the most important steps in root canal therapy (4). If the root canal system is partially cleaned, shaped and filled, success cannot be expected. Persisting bacteria in root canals may be those originally present in root canal infection that survive the biomechanical procedures and may be lodged in uninstrumented areas (5).

Root canal obturation is intended to prevent microorganisms from re-entering the root canal system, thus avoiding canal re-infection, isolate any microorganisms that may remain within the canal space from tissue fluid nutrients, thus avoiding their growth (3,4),

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and leave the tooth as most biologically inert as possible (6). It consists of extirpation of the pulp tissue and filling the space with gutta-percha cones and sealer. It is generally assumed that the amount of sealer should be minimized in relation to the gutta-percha mass. This supposition is based on reports that sealers dissolve with time and shrink during setting, thus leaving voids (7). In fact, dissolution of zinc oxide eugenol has been shown to start within 3 hours of mixing the material and continue for at least 6 months (8).

Endodontic retreatment is indicated due to prosthetic reasons or failure of the initial treatment (4,5). Prosthetic indication is usually motivated by inadequate endodontic obturation even in the absence of clinical-radiographic signs of root filling failure. Inadequate obturation may lead to coronal and apical leakage, thus exposing the root fillings to saliva and apical fluids with subsequent contamination. It has been reported that root filled teeth were completely contaminated within 19 days of bacterial coronal leakage (9).

Endodontic failure is caused by coronal leakage and persistent infection in the apical root third and/or in the periradicular tissues, even in teeth with apparently well treated canals (10). It may be attributed to complex anatomy of the root canal system and/or filling material degradation, which allows colonization of the spaces previously filled with sealer or gutta-percha.

Gutta-percha is the most widely used material for root canal filling and has been utilized for over 100 years (11). Polyisoprene (*trans*-1,4) is one of the components of gutta-percha cones, and represents 14.5 to 21.8% weight (12). The other constituents are: wax and resins (1.0 to 10.4%), ZnO (36.6 to 84.3%) and $BaSO_4$ (0 to 31.2%) (11,12). Unfortunately, degradation of root filing material may occur with some conditions, namely, temperature, light, chemical, biological and environmental factors (13-16). Aging of gutta-percha has been studied to determine its effect on mechanical properties (17) and the synergic action of aging and moisture (18). These studies evaluated commercial brands stored in different room conditions (time, temperature, humidity) to prevent degradation after root canal obturation. The process of gutta-percha degradation may be the cause of endodontic failures, making possible the loss of root filling mass and bacterial colonization.

This *in vivo* study investigated, by gel permeation chromatography (GPC) and infrared spectroscopy (FT-IR), the time of degradation of root-filling material (*trans* 1,4-polyisoprene) retrieved from endodontically treated teeth with different filling times (2 to 30 years) and correlated the occurrence of degradation with the longevity of the endodontic treatment.

MATERIAL AND METHODS

Samples

Thirty-six patients requiring nonsurgical endodontic retreatment of teeth with and without periapical lesion due to prosthetic reasons were selected from the outpatient population attending the Dental School of Fortaleza, University of Fortaleza, Brazil. The study design was approved by the institutional Ethics in Research Committee and the patients were asked to sign a written informed consent form. Detailed medical/dental history was gathered from each participant,

Teeth showing coronal leakage were excluded. Samples from 36 root canal filings aged 2 to 30 years were analyzed [teeth with (5 and 10 years) and without periapical lesion (2, 5, 8, 10, 13, 15, 18, 20, 25 and 30 years)]. Teeth with poor coronal restorations (permanent restoration with radiographic signs of overhangs, recurrent decay or open margins) were excluded (9). Information about the time of root canal filling was given by the patients. All procedures were made in triplicate.

Sampling Procedures

After access cavity preparation, rubber dam isolation and disinfection of the operative field with 2% chlorhexidine digluconate, samples of root canal fillings were collected using a Hedströem file (Dentsply/Maillefer, Ballaigues, Switzerland) without solvent. Solubilization of the samples was undertaken in chloroform (Synth, Diadema, SP, Brazil) by stirring at room temperature (~28°C) overnight. Thereafter, the mixture was filtrated in cotton filter to eliminate inorganic or insoluble materials. The solution was centrifuged (Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) at 6,000 rpm during 10 min to separate the small solid particles that remained after filtration and obtain clear solutions. GPC and FT-IR were the analytical techniques utilized. Raw gutta-percha was kindly supplied by Tanariman Industrial Ltda, Manaus, AM, Brazil. It was purified by dissolution in chloroform and precipitation with methanol (Synth).

Gel Permeation Chromatography (GPC)

The chromatographic study was performed using a liquid chromatography HPLC 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, employing toluene as the eluent and a flow rate of 1 mL/min 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, ranged from 1.13×10^3 to 2.15×10^6 g/mol.

Infrared Spectroscopy Analysis (FT-IR)

FT-IR spectra of gutta-percha film were registered in a spectrometer (8300 model, Shimadzu) in the 4000 to 400 cm^{-1} range. 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 were heated in films at 140°C in air at different times and thermal oxidation studied by FT-IR.

Statistical Analysis

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

RESULTS

The FT-IR (chromatograms) and GPC (peak molar mass and elution volume) results of root filling samples are shown in Figures 1 and 2. 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, in agreement with those values reported, in the range of 1×10^5 to 2.5×10^6 g/mol (13). A shift of peak maximum to higher elution volume with different ages of root canal filling was observed. The consequent decrease in molar mass indicates polymer degradation by polyisoprene backbone cleavage (Fig. 1).

Root fillings aged 15 years or more showed a more accentuated decrease of C=C bonds and increase of OH and C=O formation, exhibiting degradation of polyisoprene and volatile product formation, which indicates that the polymer lost weight during degradation (Fig. 2).

Degradation of *trans* 1,4-polyisoprene was significant after 15 years ($p=0.01$) in teeth without infection. The same process was observed after 5 and 10 years ($p=0.0001$) in teeth with apical periodontitis (periapical lesion).

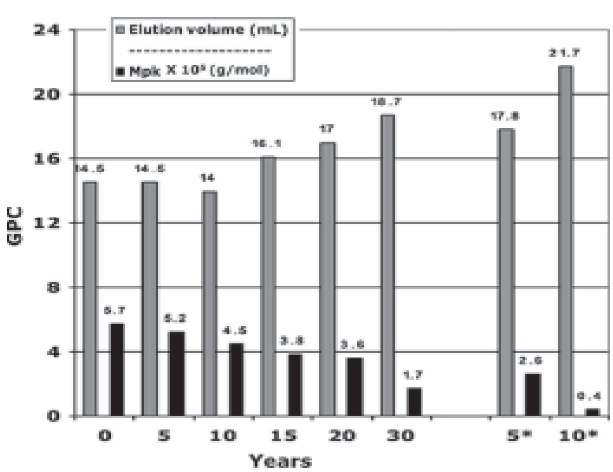

Figure 1. Graph of gel permeation chromatography (elution volume and peak molar mass) of samples retrieved from teeth with and without periapical lesion* (root canal infection).

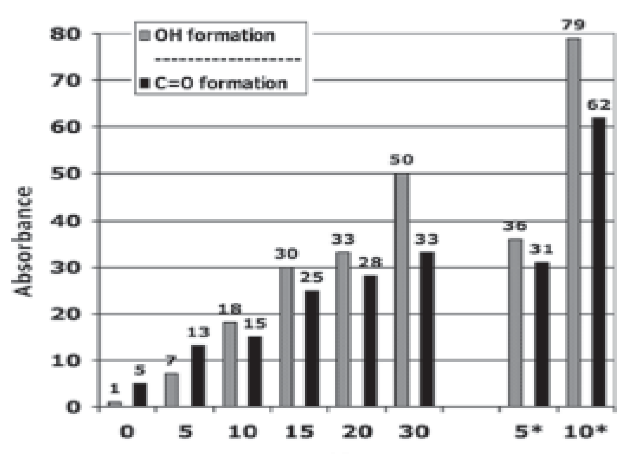

Figure 2. Graph of FT-IR spectra (OH and C=O formation) of samples retrieved from teeth with and without periapical lesion* (root canal infection).

DISCUSSION

The degradation of *trans* 1,4-polyisoprene occurs with temperature, light, chemical environmental factors (oxygen, ozone, metal) (14) and biological environmental factors (microorganisms, enzymes) (15,16). The degree of degradation seems to depend on several factors in addition to aging. In general, there is no induction period, which means that the aging process begins as soon as the teeth are treated endodontically and the gutta-percha cones are sealed inside the root canal. However, degradation was shown to be a slow and continuous process.

Degradation includes structural changes that can be analyzed by FT-IR. Up to 13 years of aging, the degradation rate is 500 times less than that of *cis* 1,4-polyisoprene film exposed to atmosphere oxygen at same temperature. After longer periods, *in vivo* gutta-percha degradation rate increases but, even though, the process is 100 times slower than that observed for the *cis* isomer degradation exposed to oxygen.

According Silva Jr. et al. (19), two main changes occur during *in vivo* aging: decrease of polymer molar mass, as an indicative of polymer backbone cleavage, and production of carboxyl (C=O) and hydroxyl (OH) groups in the residual polymer. This indicates that the aging process involves reaction with oxygen and explains the low rate, probably caused by the low availability of oxygen content within the root canal. High abnormal degree of aging was observed in infected teeth (Fig. 2 - 10* years), and suggests the participation of microorganisms in the polymer degradation (19).

Three important regions (FT-IR) could be considered after aging: i) 3400-3420 cm^{-1} attributed to OH stretching; ii) 1715-1737 cm^{-1}, due to C=O stretching; and iii) 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 correspond to products of degradation, such as alcohols, carboxylic acid, hydroperoxide, aldehyde, ketone, ether or ester, some of them present in oxidation of polyisoprene (20). The aging process includes oxidation (19).

The results of the present study showed that, after 15 years of root canal treatment, the composition of the root filling mass can be totally modified and loss of filling material may, leaving voids inside the canal system and allowing bacterial re-colonization. In these cases, the need of root canal retreatment should be evaluated. Root canal filling degradation can be the cause of endodontic failures, affecting the long-term outcomes and longevity of root canal therapy (3-5).

The decrease of molar mass occurs with aging, as expected. This fact can be attributed to a crosslink between chains. Crosslink and depolymerization are known to occur in polyisoprene degradation (14). Gutta-percha cones aged for 10 years and placed in teeth an endodontic infection showed the most aging degree (71%), which is in agreement with the results depicted on Figure 1 (10 yrs*) and Silva Jr. et al. (19).

To the best of our knowledge, only one study has already analyzed the *in vivo* degradation of root canal filling material (19). Taking into account that the *in vivo* aging is an oxidative process and could involve microbial degradation, some variables could be important, such as the presence of voids in root canal filling, apical foramen dimension or obliteration, amount and type of bacteria associated with the infection, amount of available oxygen, and coronal and apical leakage. The degradation mechanism is complex and seems to be influenced by all these variables.

The infection process makes possible microbial degradation, which is an effective way of polyisoprene degradation (15,16). Several microorganisms have been reported to degrade polyisoprene rubbers. The most common belong to actinomycetes such as *Streptomyces*, *Amycolatopsis*, and *Nocardia* sp. (16). Actinomycetes is one of the bacterial types found in root canals infection (10) that could promote *trans* 1,4-polyisoprene degradation. The biochemical and molecular basis of polyisoprene degradation is poorly understood (15). It is assumed that biodegradation of polymer backbone occurs via oxidative cleavage of double bonds (15,16), as verified in the *in vivo* aging of gutta-percha cone.

Another important effect of volatile product formation during degradation is polymer weight loss. Determination of residual weight of the *cis* 1,4-polyisoprene sample degraded by bacteria, for example, showed weight losses up to 18% for 10 weeks of incubation at 30°C (15). Even greater weight loss (80%) has been 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 (16). Weight loss in gutta-percha polymer could make the cone material more porous and reduce its root canal sealing ability.

Gutta-percha aging is a key factor on long-term success of root canal treatment, mainly because of possible migration of cytotoxic degradation products to the periodontal tissue and reduction on sealing ability caused by polymer weight loss (19). Other studies have been undertaken, seeking to correlate physical properties, chemical composition and aging degradation to the different root canal materials readily available clinicians.

The findings of the present study showed that, after 15 years, polymer weight loss may decrease the capacity of the filling mass to seal the root canal space and prevent re-infection, thus compromising significantly the longevity of root canal therapy. The degradation of *trans* 1,4-polyisoprene is an oxidative process and its degree seems to depend on several factors in addition to aging and presence of infection.

RESUMO

Este estudo *in vivo* avaliou a degradação do material obturador e a influência deste fator na longevidade do tratamento endodôntico. Foram selecionados 36 pacientes (3-30 anos) com canais tratados endodonticamente, com e sem lesões periapicais, e indicação de retratamento endodôntico. Foi investigada a associação entre o tempo de tratamento, presença de lesão periapical e a degradação do material obturador. O material obturador foi removido com uma lima Hedströem sem uso de solvente. O polímero *trans* 1,4-poliisopreno foi isolado do material obturador através de solubilização em clorofórmio, seguido de filtragem e centrifugação. GPC e FT-IR foram os métodos analíticos utilizados. A degradação do *trans* 1,4- poliisopreno foi observada com o tempo, sendo um processo lento e oxidativo, com formação de grupos carboxílicos e hidroxilas no polímero residual. Após 5 (p=0,0001) e 15 (p=0,01) anos, em dentes com e sem lesões periapicais, respectivamente, houve decréscimos significantes na massa molar do material obturador. A infecção bacteriana participa no processo de degradação do polímero. O envelhecimento da guta-percha é um fator que influencia o sucesso a longo prazo do tratamento endodôntico. Após 15 anos, a longevidade do tratamento pode ser significantemente afetada pela redução da capacidade de selamento causada pela perda de massa molar do polímero, permitindo a reinfecção do sistema de canais radiculares.

NOTE FROM THE EDITORS

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