

***In Vitro* Enzymatic Inhibition Associated with Asymptomatic Root Canal Treated Teeth: Results from a Sample of 25 Extracted Root Fragments**

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Abstract *Background:* For almost a century there has been debate over the toxicity associated with root canal treated teeth.

Objective: This study sought to determine the level of enzymatic inhibition associated with asymptomatic root canal treated teeth in which there was no radiographic evidence of pathology.

Design: In vitro study.

Setting: Private dental office (Marble Falls, Texas, USA).

Intervention: A photo-affinity labeling technique performed by Affinity Labeling Technologies (ALT) Bioscience Laboratory was used to determine the level of enzymatic inhibition of the extracted teeth.

Main outcome measures: Six commercially available enzymes that are critically important for human life were tested in order to determine their percent inhibition when exposed to root fragments of the 25 extracted root canal treated teeth.

Results: The enzymes averaged 65.6% inhibition, which reflected severe toxicity on the scale developed by ALT Bioscience Laboratory. Limitations of this study included the lack of a control group, the inability to ascertain if these in vitro results have significant clinical relevance, the inability to correlate these in vitro findings to a patient's clinical health status, possible contamination of the root fragments with mercury or other substances, and the absence of culturing the root fragments.

Conclusion: Root canal therapy has proved to be an effective way of treating and retaining endodontically diseased teeth, but does not render the teeth sterile. These preliminary results suggest that root canal treated teeth inhibit the action of critically important enzymes that generate cellular adenosine triphosphate.

Introduction

For almost 100 years there has been debate regarding toxicity associated with teeth that have undergone root canal therapy. Weston Price, D.D.S., M.S., F.A.C.D., Director of the Research Institute of the National Dental Association, published an exhaustive 1,044 page, two-volume treatise in

1923, which correlated numerous systemic illnesses with diseased teeth, including teeth that had undergone root canal therapy. His testing methods involved isolating bacteria from infected teeth, injecting these bacterial cultures into experimental animals, and then observing the systemic effects on the experimental animals. He found that many sys-

temic illnesses were precipitated by diseased teeth, including teeth which had undergone root canal therapy.^{1,2}

Recent research is consistent with Price's findings and has revealed that teeth which have undergone root canal therapy continue to harbor bacteria, viruses, and fungi.³⁻¹¹ Unpublished research by Nunnally has revealed via deoxyribonucleic acid analysis that endodontically treated teeth play host to numerous species of anaerobic bacteria. The toxicities associated with endodontically treated teeth do not remain silently tucked away within the structure of the dead tooth.

This study sought to determine the level of enzymatic inhibition associated with root canal treated teeth which: (1) had undergone root canal therapy as confirmed by radiographs; (2) had no radiographic evidence of disease; and (3) produced no symptoms, i.e., teeth were asymptomatic with respect to chewing pressure or thermal sensitivities and were indistinguishable by the patient from any of the patient's other non-root canal treated teeth.

Methods

During a three year period, 87 root canal treated teeth were extracted on consecutive patients who requested removal of their asymptomatic root canal treated teeth. After root canal therapy, all of these patients noted declines in their overall health and had been informed by their health care providers of the possible systemic health risks associated with root canal treated teeth. Only extracted root canal treated teeth were accepted into the study.

Radiographs of the 87 extracted teeth were evaluated by Nunnally and independently evaluated by three independent dentist evaluators who were asked to critically examine each radiograph. Radiographs of the teeth prior to endodontic treatment were not evaluated. Only teeth which radiographically had been completely obturated to within 0.5 millimeters of the apex, had no extrusion of the root canal filling material, and which showed no signs of disease were admitted into the study.

Of the 87 original teeth, 25 were unani-

mously approved by Nunnally and three independent dentist evaluators for inclusion in this study. Of the 25 teeth, 15 had been treated by endodontists, six had been treated by general dentists and in the remaining four teeth, the patients were unable to recall who performed the root canal therapy. The included teeth consisted of twelve molars, eight bicuspid, three canines, and two incisors. All 25 teeth appeared radiographically and clinically to have been filled with gutta percha, but no attempt was made to determine which endodontic sealer had been used.


The extracted teeth were placed into sterile biopsy bottles and submitted to Affinity Labeling Technologies (ALT) laboratory for in vitro toxicity testing. Root fragments were analyzed from the 25 extracted teeth containing restorative materials, such as mercury fillings, metallic, ceramic, or metaloceramic crowns. The individual root fragments were carefully selected to minimize the impact of contamination or leaching from the restorative materials. The ALT *in vitro* toxicity testing was performed on all root fragments after washing them three times in distilled water, and, after filtration, 10 microliters of each third wash sample was incubated with six enzymes (i.e., phosphorylase kinase, phosphorylase A, pyruvate kinase, phosphoglycerate kinase, creatine kinase, and adenylate kinase) to determine the degree of enzymatic inhibition. The degree of enzymatic inhibition for the root fragments was quantified by using a nucleotide photo-affinity labeling technique.

No control samples were submitted for comparison since that would have required the extraction of healthy, non-root canal treated teeth from patients whose teeth were included in this study.

Results

The results for all root fragments from the 25 extracted root canal treated teeth were calculated. Figure 1, (p. 114) provides a sample report from one of the collected specimens. The mean percent of total enzymatic inhibition was calculated from 150 results (six enzyme percent scores X 25 specimens = 150) and determined to be $65.6\% \pm 16.6$.

Figure 1. ALT *in vitro* toxicity testing results for one of the root fragments derived from an extracted root canal treated tooth



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REPORT OF IN VITRO SAMPLE TOXICITY
 (Assessed by nucleotide photoaffinity labeling of purified enzymes treated with sample extract)

Patient		Doctor	
Name:		Name:	Dr. Stuart Nunnally, DDS
Sex: F DOB:		Address:	2100 Hwy 1431
Sample:	Extracted Tooth # 3		Marble Falls, TX 78654
ALT#:		Phone:	(830) 693-3646
Date Sample Analyzed:	1/11/08	FAX:	(830) 693-4061

Enzyme Assayed	% Inhibition	Toxicity Level	0	1	2	3	4	5
Phosphorylase Kinase	81.4%	5 = extreme						X
Phosphorylase A	79.5%	5 = extreme						X
Pyruvate Kinase	72.9%	4 = severe					X	
Phosphoglycerate Kinase	65.3%	4 = severe					X	
Creatine Kinase	75.0%	4 = severe					X	
Adenylate Kinase	54.8%	4 = severe					X	
Average	71%						X	

Toxicity Scale

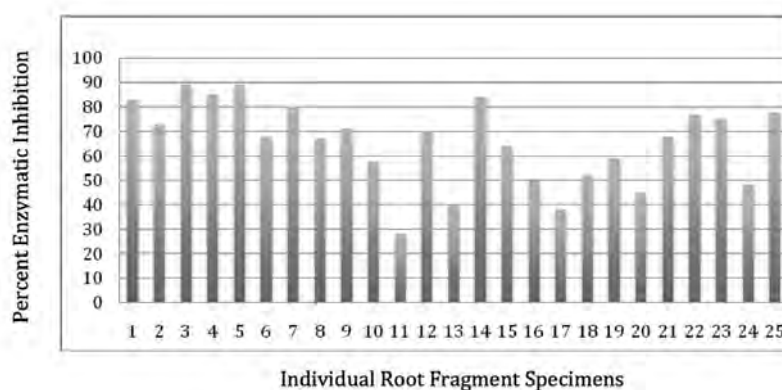
0	< 5% inhibition, no observable toxicity to these particular enzymes though others may be adversely affected
1	5-20% inhibition, slight toxicity
2	21-35% inhibition, mild toxicity
3	36-50% inhibition, moderate toxicity
4	51-75% inhibition, severe toxicity
5	>75% inhibition, extreme toxicity

Comments:

Date results reported: 1/16/08

Sample analyzed by: MC

Figure 2. Calculated enzymatic inhibition for individual root fragments derived from 25 extracted root canal treated teeth



The percent of enzymatic inhibition for all collected specimens ranged from a low of 28% to a high of 89%, with a median of 68%. (Figure 2, p. 114).

Discussion

Since no control group was used, it is difficult to know if these *in vitro* results have significant clinical relevance. It is impossible to know if root fragments from properly matched non-root canal treated teeth would produce less enzymatic inhibition compared to root fragments derived from root canal treated teeth. It is also difficult to correlate these *in vitro* findings to a patient's clinical health status. It is also possible that the root fragments were contaminated by mercury or other substances even though samples were carefully chosen to minimize these effects. None of the root fragments were analyzed or cultured. These results, therefore, can only be considered very preliminary in nature. More studies are certainly needed, which must include a control group, a more precise analysis of the root fragments, and the ability to better correlate a patient's clinical health status to root canal treated teeth and to *in vitro* enzymatic inhibition.

Notwithstanding the significant limitations of this study, there is an increasing awareness among health care providers and the general population that infected teeth and periodontal disease can have profound systemic implications. For example, periodontal disease has been cited as one of the greatest influences in predicting a stroke.¹¹⁻¹³ Infected teeth have been linked to cancer, brain and lung abscesses, heart disease, disorders of the eyes, sinuses, digestive tract and virtually every other systemic organ.¹⁴⁻¹⁹ Price demonstrated repeatedly that autoimmune diseases such as rheumatoid arthritis could be caused or exacerbated by infected teeth.^{1,2} Anecdotes abound of people who have had an infected tooth extracted only to have their joint pain "magically" disappear within days.

There is also a general misperception among dental practitioners that root canal treated teeth are devoid of pathogens and

toxins. The bacteria which reside within the tubules and accessory canals of root canal treated teeth (there are an estimated three miles of untreated microscopic tubules in a single rooted incisor²⁰) become producers of potent toxins. Thus, the working (i.e., unproven) hypothesis is that the bacteria and toxins within root canal treated teeth inhibit critically important enzymes essential for human life, which leads to negative health outcomes. The enzymes tested for inhibition by ALT Laboratory are necessary for life because they are associated with the cell's production of adenosine triphosphate (ATP).

Dentists have assumed that the success of root canal treatment is measured in terms of longevity of the treated teeth, yet the findings of Price and others and these preliminary results suggest concerns over the possible systemic effects associated with root canal treated teeth.

Conclusion

Root canal therapy has proved to be an effective way of treating and retaining endodontically diseased teeth, yet research has demonstrated that root canal therapy does not render the tooth sterile. These preliminary results suggest that root canal treated teeth inhibit the action of critically important enzymes that generate cellular ATP.

Competing Interests

The author declares that he has no competing interests.

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