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The ability of thiourea to scavenge hydrogen peroxide and hydroxyl radicals during the intra-coronal bleaching of bloodstained root-filled teeth

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Abstract

Background: Hydrogen peroxide, an agent used in the intra-coronal bleaching of root-filled teeth for over a century, has been shown to diffuse from the pulp chamber to the outer root surface. Furthermore, it has been demonstrated that destructive hydroxyl radicals, the by-products of the bleaching process, have been detected on the external root surface. The control of such diffusion may be of importance in minimizing the risk of invasive cervical resorption (ICR) which has been linked to intra-coronal bleaching of discoloured root-filled teeth using hydrogen peroxide. The aims of the present *in vitro* study are to quantify the diffusion of hydrogen peroxide and hydroxyl radicals to the outer root surface following intra-coronal bleaching, and to evaluate the ability of thiourea incorporated into the bleaching protocol to scavenge residual hydrogen peroxide and hydroxyl radicals.

Methods: Thirty-five single rooted premolar teeth with intact cementum at the cemento-enamel junction were used in this project. Thirty teeth were stained with red blood cells and root-filled with gutta-percha and AH26. The five unstained teeth were root-filled and constituted a negative control (Group 1). The stained teeth were divided equally into the following experimental groups and subjected to various intra-coronal bleaching regimes: Group 2 – 'walking bleach' with 20µl 30 per cent w/w hydrogen peroxide; Group 3 – 20µl 30 per cent w/w hydrogen peroxide and thermocatalytically activated; Group 4 – 20µl acidified thiourea; Group 5 – 20µl acidified thiourea and 20µl 30 per cent w/w hydrogen peroxide; Group 6 – 20µl acidified thiourea and 20µl one per cent sodium hypochlorite; Group 7 – 20µl acidified thiourea, 20µl one per cent sodium hypochlorite and 20µl 30 per cent w/w hydrogen peroxide. The reaction products of the bleaching process were quantified at the outer root surface using high performance liquid chromatography and electrochemical detection (HPLC-ECD).

Results: Results showed that hydrogen peroxide used alone in Groups 2 and 3 was able to be detected at the outer root surface in 100 per cent of the samples, and that the presence of the hydroxyl radical generated in both groups was detected in equal amounts ($P<0.05$). When thiourea was incorporated into the bleaching protocols in Groups 5–7, it was shown to scavenge both hydrogen peroxide and hydroxyl radicals to a significant degree ($P<0.05$).

Conclusions: Acidulated thiourea is an effective scavenger of residual hydrogen peroxide and hydroxyl radicals generated during the intra-coronal bleaching of bloodstained root-filled teeth.

Key words: Thiourea, hydrogen peroxide, hydroxyl radicals, intra-coronal bleaching.

Abbreviations and acronyms: ICR = invasive cervical resorption; HPLC = high performance liquid chromatography; ECD = electrochemical detection; 2,5-DHB = 2,5-dihydroxybenzoic acid; 2,3-DHB = 2,3-dihydroxybenzoic acid; EDTA = ethylenediaminetetracetic acid; NaOCl = sodium hypochlorite; 3,4-DHB = 3,4-dihydroxybenzoic acid; CEJ = cemento-enamel junction.

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INTRODUCTION

The intra-coronal bleaching of bloodstained root-filled teeth has been an accepted clinical procedure in dentistry for over 100 years,[1] and currently the most common techniques utilize hydrogen peroxide as the bleaching agent. A widely used method introduced by Nutting and Poe[2] in 1963 is known as the 'walking bleach' – a technique which relies on the inherent instability of 30 per cent w/w hydrogen peroxide to decompose into its oxidizing components and to penetrate into radicular dentine, and bleach stained dentine. In order to improve the effectiveness of the bleaching process, the same authors mixed sodium perborate with 30 per cent w/w hydrogen peroxide and sealed the slurry into the pulp chamber. This technique is called the 'combination walking bleach', and this method is still practised today.[3] The activation of

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hydrogen peroxide by a photoflood or ultraviolet lamp or a heated instrument has been advocated since the early twentieth century and, in 1965, Stewart labelled the technique 'thermocatalytic bleaching'.[4]

The first association between the use of intra-coronal bleaching of traumatized root-filled teeth with hydrogen peroxide and a form of external root resorption was reported in 1979.[5] Several other case reports followed.[6-8] This insidious form of external root resorption, termed invasive cervical resorption, is most commonly seen at the level of the cemento-enamel junction.[9] Reports of an association between invasive cervical resorption and intra-coronal bleaching has resulted in alternative bleaching protocols being proposed. Some authors caution against the use of heat to activate hydrogen peroxide.[7,10] Others recommend that hydrogen peroxide be replaced by a mixture of sodium perborate and water.[8]

Marin *et al.*[11] demonstrated that the staining of the traumatized tooth was due to the release of haemoglobin and haem moieties which pass into the dentinal tubules. Hydrogen peroxide is capable of reacting with available iron found associated within haemoglobin and located within intratubular dentine of bloodstained teeth to produce hydroxyl radicals. Both hydrogen peroxide and hydroxyl radicals are capable of damaging cells and tissues of the periodontal ligament. The hydroxyl radical is extremely reactive and is highly toxic to fibroblasts, the major cell type in the periodontal ligament. Its role in the destruction of connective tissue components, collagen and hyaluronic acid is well documented.[12] Dahlstrom *et al.*[13] reported on the generation of hydroxyl radicals on the outer surface of teeth that had been stained with blood and thermocatalytically bleached. In the same study, hydrogen peroxide was detected on the outer root surface in a small percentage of the samples. This latter finding was in agreement with Rotstein *et al.*[14] who detected hydrogen peroxide on the outer root surface that had been stained with blood and intra-coronally bleached.

Thiourea has been used in the textile industry for many years as a reducing agent to bleach wool and paper. In the medical field, thiourea has been used to scavenge hydroxyl radicals and a number of other oxygen derived free radicals during the reperfusion of various organs and in the treatment of various disease states.[15] A recent study showed that the introduction of thiourea into the intra-coronal bleaching of blood-stained teeth had no detrimental effect on the bleaching efficacy of hydrogen peroxide and its introduction may allow for the scavenging of hydrogen peroxide and hydroxyl radicals.[16]

Other oxygen radical scavengers have been investigated for their potential use in the intra-coronal bleaching of bloodstained root-filled teeth. Rotstein[17] introduced catalase into the pulp chamber after 60 minutes of bleaching with hydrogen peroxide and found it to be an effective scavenging agent.

The present *in vitro* study aimed to quantify the diffusion of hydrogen peroxide and hydroxyl radical production to the outer root surface following intra-coronal bleaching, and to evaluate the ability of thiourea, incorporated into the bleaching protocol, to scavenge residual hydrogen peroxide and hydroxyl radicals.

MATERIALS AND METHODS

The presence of hydrogen peroxide and hydroxyl radicals was determined by the detection of the reaction products with a test solution of sodium salicylate using high performance liquid chromatography and electro-chemical detection (HPLC-ECD).

HPLC assay for hydroxyl radical formation

The salicylate hydroxylation assay was used to detect hydroxyl radical formation in the bathing solution of control and treated teeth. The method is based on the detection of various hydroxylated derivatives of salicylate formed during hydroxyl radical trapping reactions (2,5-dihydroxybenzoic acid (2,5-DHB), 2,3-dihydroxybenzoic (2,3-DHB) and pyrocatechol) via HPLC with electrochemical detection (ECD). Since hydrogen peroxide and thiourea are also electro-chemically active, the use of the ECD device also allowed detection of any of these reagents that diffused from the pulp chamber into the bathing solution.

The HPLC system comprised a GBC LC 1150 reverse phase HPLC pump. This pump was equipped with an ERC-3415 membrane-type degasser. The electro-chemical detector was a Bioanalytical Assay Systems (BAS) LC-4A Amperometric Detector. Output from the detector was collected using Delta Junior chromato-graphy analysis software (Version 5009, Digital Solutions, Queensland). The glassy carbon electrode of this device was set at an oxidation potential of +0.80V against the Ag/AgCl working electrode. This oxidation potential was shown by Dahlstrom *et al.*[13] to provide sufficient sensitivity with minimal background noise under similar experimental conditions. The mobile phase comprised 70 per cent aqueous phase and 30 per cent methanol, with the former containing 0.1M potassium di-hydrogen orthophosphate (KH_2PO_4) and 0.1m EDTA prepared in milliQ water. The pH of the aqueous phase was adjusted to (pH = 2.9) prior to use. The mobile phase was pumped at a 1ml/min flow rate through a C-18 column.

Preparation of teeth

The teeth used in the research project were extracted as part of orthodontic treatment requirements and approval was received from the Human Ethics Committee of The University of Adelaide for the use of such teeth.

Thirty-five single canal premolar teeth were collected and stored in phosphate buffered saline with added thymol (an anti-fungal agent) until they were to be used. The experimental teeth used in this study were selected because they were anatomically similar and

appeared to have intact cementum at the cemento-enamel junction. This was determined by microscopic examination using a Wild Heerbrugg M400 stereo-microscope at seven times magnification. The periodontal ligament was removed from the outer surface of the tooth by gentle rubbing of the outer surface of the tooth with gauze soaked in sterile isotonic saline. No chemicals that could cause contamination and subsequently affect the assay for hydroxyl radicals were used on the external root surface. The teeth were re-examined under magnification to ensure complete removal of the periodontal ligament had occurred. Occlusal access cavities were cut into the teeth using a high speed tungsten carbide bur and the pulp tissue was extirpated from the canal of each of the teeth using endodontic files. The canals were debrided to a size 35 master apical file. The canals were irrigated with sterile saline throughout the debridement process. Sodium hypochlorite was not used throughout the debridement of the root canals to eliminate the potential contamination of the test results with residual sodium hypochlorite.

Thirty teeth were placed crown first into 10ml polyethylene centrifuge tubes and the tubes were filled with 6ml of the packed red blood cells, completely immersing the teeth. Discolouration of the teeth occurred by using a modified version[11] of a technique initially devised by Freccia *et al*.[18] The other five teeth remained unstained and formed the negative control group (Group 1).

Following visible discolouration, the teeth were removed and irrigated with sterile saline before being obturated with gutta-percha and AH26® sealer cement (De Trey, Zurich, Switzerland) using a lateral condensation technique. The obturation was finished at a level 1mm below the CEJ and this was confirmed by radiographic examination. The pulp chambers of the canals were swabbed with 15 per cent ethylenediaminetetracetic acid (EDTA) to remove excess sealer and then rinsed thoroughly with sterile saline to remove any traces of the EDTA solution. Each tooth was set into a 1mm thick sheet of modelling wax at a level 2mm above the cemento-enamel junction in a manner similar to Dahlstrom *et al*.[13] This assembly was then suspended in a 2.5ml polycarbonate assay tube filled with the 1mM sodium salicylate solution dissolved in water.

The teeth were divided into seven experimental groups of five teeth each: Group 1: Unstained teeth – empty pulp chamber (negative control); Group 2: Bloodstained teeth – bleached with 20µl 30 per cent w/w hydrogen peroxide – 'walking bleach technique'; Group 3: Bloodstained teeth – thermocatalytically bleached with 20µl 30 per cent w/w hydrogen peroxide; Group 4: Bloodstained teeth – bleached with 20µl acidified thiourea (pH = 2.95); Group 5: Bloodstained teeth – bleached with 20µl acidified thiourea (pH = 2.95) and 20µl 30 per cent w/w hydrogen peroxide; Group 6: Bloodstained teeth – bleached with 20µl acidified thiourea (pH = 2.95) and 20µl one per cent sodium hypochlorite (NaOCl); Group 7: Bloodstained teeth – bleached with 20µl acidified thiourea (pH = 2.95), 20µl one per cent sodium hypochlorite and 20µl 30 per cent w/w hydrogen peroxide.

Each test solution was introduced by pipette onto a sterile cotton pledget placed in the pulp chamber. In Group 3, the hydrogen peroxide was activated with a red-hot ball burnisher that had been heated in a blue flame. The heat was applied for 10 second intervals and was repeated five times over a 20 minute period. Test Groups 5–7 each had 20µl of the test solutions pipetted into the pulp chamber of the tooth in the order in which they are listed above. Spectrophotometric analysis confirmed that the 30 per cent hydrogen peroxide was at the correct concentration. The access cavity of each tooth was sealed with 2mm of Cavit® (Espe, Seefeld, Germany) immediately following placement of the test solution. Following the bleaching procedure, the teeth were stored at 37°C in an incubator chamber for 48 hours before samples of each salicylate bath were tested.

Two samples of each test solution were analysed in succession so that test conditions for both samples were as identical as possible. To the first sample was added a 20µl sample of the internal standard: 2µM solution of 3,4-dihydroxybenzoic acid (3,4-DHB). The second sample, injected onto the column immediately after the first sample had passed across the column, did not have the internal standard present to ensure that contamination of the internal standard had not occurred. The amounts of each of the reaction products and of hydrogen peroxide and thiourea were measured as picomoles/nanomoles per 20µl sample. Statistical analysis of the raw data was performed using two-tailed Student *t* tests to analyse the significance of difference between groups for the quantities of each of the test solutions.

RESULTS

The results of these sensitivity assays showed that the practical limit of sensitivity for the 2,3-DHB, 2,5-DHB and the 3,4-DHB to be 800 femtomoles. The practical limit of the sensitivity was 1 picomole for thiourea and 30 nanomoles for hydrogen peroxide. The sensitivity of the HPLC-ECD and the test conditions were considered standardized for the determination of the quantity of hydroxyl radical production. A total of 35 teeth were sampled over a period of 15 hours and the generation of amounts of 2,5-DHB was measured in picomoles. The generation of hydrogen peroxide and thiourea was measured in nanomoles. The amount of each of these reaction products present in the salicylate bath, and therefore the amount of hydroxyl radical generated on the outer surface per tooth, could be calculated by the following equation:

$$\frac{\textit{No. of picomoles in sample injected onto column} \times \textit{(Volume of salicylate bath} \times 10^3\textit{)}}{\textit{Volume of sample}}$$

Table 1. Average quantity of each of 2,5-DHB, hydrogen peroxide and thiourea found in the test solution samples, represented as picomoles or nanomoles per tooth

Sample group	Solution	2,5-DHB (average pmol)	Hydrogen Peroxide (average nmol)	Thiourea (average nmol)
Group 1	Negative Control	0	0	0
Group 2	Hydrogen Peroxide 'Walking Bleach'	591	205047	0
Group 3	Hydrogen Peroxide 'Thermocatalytic'	913	63092	0
Group 4	Thiourea	0	0	317
Group 5	Hydrogen Peroxide and Thiourea	173	22401	179
Group 6	Sodium Hypochlorite and Thiourea	132	0	119
Group 7	Hydrogen Peroxide, Sodium Hypochlorite and Thiourea	212	20649	59

The relationship between the number of picomoles of 2,5-DHB and the number of nanomoles of hydrogen peroxide and thiourea for each of the test groups is shown in Table 1.

Group 1 did not show the presence of any end products of the hydroxylation of salicylate by hydroxyl radicals. No hydrogen peroxide or thiourea was detected indicating there was no contamination of the control tooth samples. The amount of the reaction product 2,5-DHB for each of the test group are outlined in Fig 1. The generation of 2,5-DHB in the salicylate bath was greatest for Group 3 where an average of 912.6pmol was detected in the samples. This was greater than that detected in Group 2 (mean = 739.9pmol). The average number of picomoles detected in Group 5 was significantly lower than that found in Group 2 ($P<0.05$), but not significantly lower than that in Group 3 ($P = 0.19$). There was no significant difference in the generation of 2,5-DHB between Groups 2 and 3 ($P = 0.78$).

The amount of hydrogen peroxide that was detectable in each group can be seen in Fig 2. Group 2 samples provided the greatest quantities of hydrogen peroxide (20504 nmol/tooth), which was significantly greater ($P<0.05$) than the number of nanomoles detectable in any of the groups containing thiourea. There was a statistically significant decrease in the amount of detectable hydrogen peroxide in Group 3 compared to Group 2 ($P = 0.03$). This decrease would be expected as the heat catalyses the breakdown of hydrogen peroxide.

The detectable quantities of thiourea present in the sample groups are shown in Fig 3. Thiourea was detected in 19 of 20 (95 per cent) samples into which it was introduced. Group 4 samples provided the greatest quantities of thiourea on the outer surface of the tooth. The mean number of nanomoles in Group 4 was 317nmol. This was significantly greater than the number of nanomoles of thiourea found on the outer surface of Groups 5–7 ($P<0.05$), which had mean values of 179nmol, 119nmol and 58.7nmol respectively. The least amount of detectable thiourea was in Group 7 when both hydrogen peroxide and sodium hypochlorite were present in the pulp chamber and therefore, a greater amount of thiourea was consumed during the scavenging of hydrogen peroxide and hydroxyl radicals within the dentinal tubules.

The presence of sodium hypochlorite in the pulp chamber produced small amounts of hydroxyl radicals on the outer surface of the teeth in Group 6.

DISCUSSION

The degree of sensitivity demonstrated by the HPLC-ECD in this research was found to be in accordance with that found by Dahlstrom *et al.*[13]

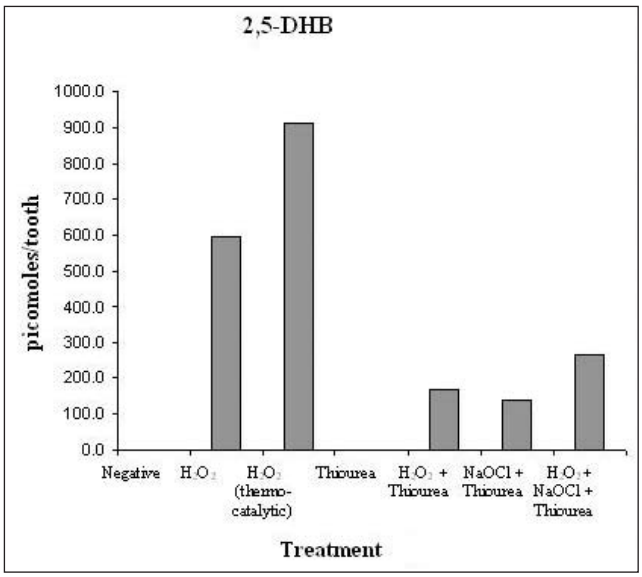

Fig 1. Average number of picomoles/tooth of 2,5-DHB detected in the salicylate bath for each sample ($P<0.05$).

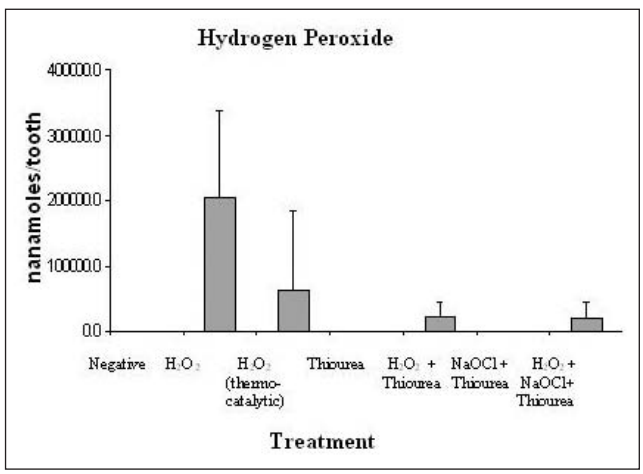

Fig 2. Average number of nanomoles/tooth of hydrogen peroxide detected in the salicylate bath for each sample group ($P<0.05$).

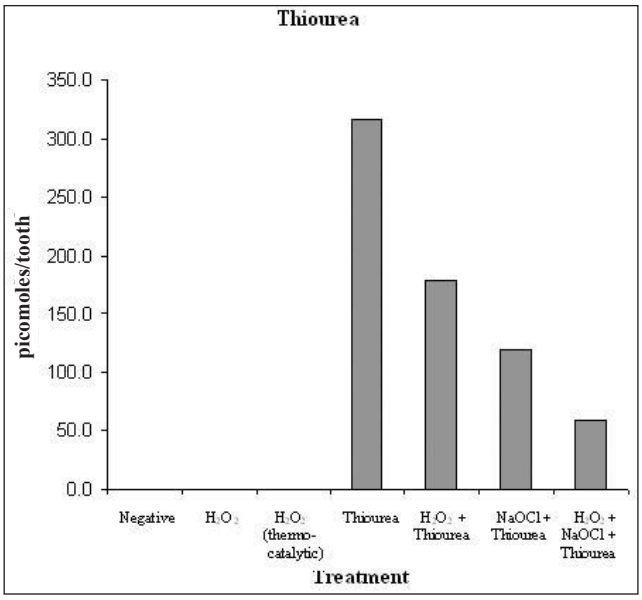

Fig 3. Average number of picomoles/tooth of thiourea detected in the salicylate bath for each test group ($P<0.05$).

The application of heat to activate hydrogen peroxide in the pulp chamber has been advocated. However, the application of heat had two effects: firstly, it catalyses the breakdown of the hydrogen peroxide into its unstable oxidizing components; and secondly, it imparts energy to the bleaching solution, which may cause it to diffuse more effectively into the dentine tubules of the stained tooth structure.[19,20] It has been demonstrated that increasing the temperature of radicular dentine by 40°C will result in hydraulic conductance of non-etched dentine increasing 1.8-fold and fourfold in etched dentine.[21] This has been supported by other studies which have shown an increase of 10°C almost doubled dentine permeability.[22] This would explain the lesser amounts of hydrogen peroxide detected on the outer surface of the tooth in teeth that were activated by heat. However, there was an increased amount of hydroxyl radicals detected in the outer layers of radicular dentine and on the outer surface of the tooth in this group. Hydrogen peroxide not immediately broken down into its unstable oxidizing components would be capable of more readily diffusing through the expanded dentinal tubules towards the outer surface of the root. Likewise, salicylate would be more readily able to diffuse into the tooth to react with the hydroxyl radicals produced in the outer layers of the dentine. These reaction products would then be free to diffuse out of the tooth into the salicylate bath and be detected. As the inward diffusion of tissue fluids would not be expected in the *in vivo* situation, this may suggest a possible limitation of the experimental model in the current research. The increased generation of hydroxyl radicals detected in the raw data in Group 3 suggests the possibility that heat activated hydrogen peroxide produces more hydroxyl radicals on the outer root surface, and therefore should not be used during the intra-coronal bleaching of root-filled teeth.

The ability of thiourea to scavenge both hydroxyl radicals and hydrogen peroxide has been well documented.[15] Its use as a scavenger of oxygen radicals and reactive oxygen species has been used for many years in medical practice. However, its use in dentistry and, in particular for the intra-coronal bleaching of root-filled teeth, is novel. The average amount of 2,5-DHB detected within the salicylate bath per tooth in Groups 5 and 7 was significantly less than in Groups 2 and 3. In this research project, the introduction of thiourea has therefore been shown as an effective scavenger of hydroxyl radicals and hydrogen peroxide in the radicular dentine.

Thiourea was found to pass from the pulp chamber to the outer root surface of the tooth in 19 (95 per cent) of the 20 teeth. The amounts present were directly proportional to the scavenging effect the thiourea had on the hydroxyl radicals and hydrogen peroxide. The reported LD_{50} (50 per cent lethal dose) for thiourea is 1830mg/kg.[23] This reported LD_{50} is important when considering the amount of thiourea passing to the outer root surface of the tooth. Conversion of the number of nanomoles of thiourea present on the outer root surface to the number of milligrams of thiourea present per tooth reveals that for Group 4 the amount of thiourea present was 8.86mg per tooth. This value decreases to 5.1×10^{-3}mg per tooth for Group 5 and to 1.66×10^{-3}mg per tooth for Group 7. Therefore, the levels of thiourea detected were not deemed toxic and were well below the determined LD_{50} for thiourea.

Sodium hypochlorite was included in the bleaching protocol of Groups 6 and 7 to aid in the removal of residual organic debris from within the pulp chamber prior to placement of the bleaching agent. Residual organic debris within the pulp chamber will impede the efficacy of the bleaching agent. The results from Group 6 indicated that sodium hypochlorite is capable of generating hydroxyl radicals. The amount of detectable hydroxyl radicals on the outer root surface was significantly less for Group 5 than Group 6. This may be explained by the ability of thiourea to scavenge hydrogen peroxide and, therefore, limit the amount of hydroxyl radical production on, or close to the outer root surface. However, thiourea may have a lesser capacity to scavenge sodium hypochlorite allowing greater volumes to diffuse through to the root surface. The significant effect of the introduction of sodium hypochlorite on the production of hydroxyl radicals on the outer root surface suggests that the efficacy of this irrigant in the removal of residual organic debris from the pulp chamber may be outweighed by its capacity to generate hydroxyl radicals and therefore, should not be used in the intra-coronal bleaching of bloodstained root-filled teeth.

The passage of hydrogen peroxide through dentine was a significant finding. The results indicated that whenever hydrogen peroxide was introduced into the pulp chamber significant amounts were detectable on the outer root surface after 48 hours. The results in

Group 2 indicate that the presence of intact cementum at the level of the CEJ and along the root surface did not impede the passage of hydrogen peroxide. The results of the present research may imply that the gutta-percha and sealer do not act as a protective barrier to the penetration of hydrogen peroxide down the root canal. The use of a 2mm Cavit® base may explain why a smaller percentage of the sample group in this study exhibited hydrogen peroxide on the outer root surface. The use of a base is advocated by several authors.[24-29] These authors proposed that the use of a base prevented or decreased the penetration of hydrogen peroxide to the outer root surface of the tooth. These authors showed that the use of at least 2mm of a base material was an effective barrier to the penetration of hydrogen peroxide along the length of the root canal. Dahlstrom *et al*.[13] found that in only five of 40 teeth (12.5 per cent) bleached with hydrogen peroxide was it able to be detected on the outer root surface. This supports the argument for the use of a base in the intra-coronal bleaching of root-filled teeth. However, other authors contend that there is no base that prevents percolation of hydrogen peroxide through the root filling.[30] Further research into the type of base and the ability of each base to inhibit the passage of hydrogen peroxide and hydroxyl radicals is warranted.

Fibroblasts are the predominant cell type in gingival and periodontal tissue. They are responsible for the maintenance and integrity of these tissues. The ED_{50} (dose at which 50 per cent of cells are inhibited from functioning) value for hydrogen peroxide has been determined to be between 5.0–10.0mmol/litre.[31] The conversion of the number of nanomoles of hydrogen peroxide per 20ml sample of salicylate to the number of millimoles of hydrogen peroxide per millilitre of salicylate (mmol/ml) allows determination of the average amount of hydrogen peroxide able to diffuse to the outer root surface. Data from Group 2 shows that 75mM of hydrogen peroxide diffused to the outer root surface. This value is above the ED_{50} value for hydrogen peroxide, and therefore consideration needs to be given to the use of hydrogen peroxide alone if the current test conditions were to be used *in vivo*. The average concentration of hydrogen peroxide detected in Group 5 samples was 8mM and the average quantity of hydrogen peroxide detected in Group 7 was 7.7mM. These values are found within the ED_{50} value range for hydrogen peroxide and are acceptable levels of hydrogen peroxide to be passing through the radicular dentine from the pulp chamber to the outer root surface. The inclusion of thiourea into the bleaching protocol, whilst not eliminating the passage of hydrogen peroxide to the outer root surface, reduces the potential for damage to the major cell type of the periodontal ligament.

The initial insult to the periodontium responsible for the onset of the resorption process following intra-coronal bleaching is hypothesized as being the presence of hydroxyl radicals. These radicals are formed by a Fenton reaction between hydrogen peroxide and ferrous (Fe^{2+}) ions. A question arises as to whether it is necessary to counteract hydroxyl radicals before they can institute the level of damage necessary to trigger such a resorptive response. The association between intra-coronal bleaching and invasive cervical resorption has been well documented.[5,6,7,10] The ability of hydroxyl radicals to cause damage to elements of the periodontal ligament at the cellular and non-cellular level, which may result in necrosis of this tissue, has been established as has the correlation between necrotic periodontal ligament and root resorption.[32] The presence of hydroxyl radicals on the outer root surface of the tooth are capable of causing necrosis of the periodontal ligament or impairing the normal function of this tissue, thereby offering a possible cause for the initiation of the form of external root resorption known as invasive cervical resorption. In the current research project, the hydroxyl radicals generated as a result of the reaction between hydrogen peroxide and the available ferrous (Fe^{2+}) iron occurs in the first 30 minutes. The advantage of thiourea is that it is introduced into the tooth prior to the introduction of hydrogen peroxide and acts as a scavenger of both hydrogen peroxide and hydroxyl radicals in the intra-coronal bleaching of bloodstained root-filled teeth.

CONCLUSIONS

Both the 'walking' and 'thermocatalytic' bleaching techniques are capable of generating hydroxyl radicals on the outer root surface of the tooth, although the thermocatalytic bleaching technique produced more hydroxyl radicals on the outer root surface than the walking bleach technique. Hydrogen peroxide was capable of diffusing through radicular dentine to the outer root surface of the teeth in all groups into which it was introduced.

Thiourea was capable of significantly reducing, but not totally eliminating, the quantities of hydrogen peroxide and hydroxyl radicals detectable on the outer root surface of the tooth. When thiourea was not used in the bleaching protocol the amount of hydrogen peroxide capable of diffusing through radicular dentine was above the LD_{50} for hydrogen peroxide. However, when thiourea was introduced into the bleaching protocol the amount of hydrogen peroxide capable of diffusing through radicular dentine was below the LD_{50} for hydrogen peroxide. The inclusion of thiourea in the intra-coronal bleaching of bloodstained root-filled teeth is recommended.

The development of a combined oxidative–reductive bleaching protocol for root-filled teeth using hydrogen peroxide and thiourea offers the possibility of producing a safer bleaching regimen without interfering with the bleaching efficacy of the hydrogen peroxide.

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