The effect of German chamomile (*Marticaria recutita* L.) extract and tea tree (*Melaleuca alternifolia* L.) oil used as irrigants on removal of smear layer: a scanning electron microscopy study

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Abstract

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Aim To compare the cleaning effectiveness of chamomile hydroalcoholic extract and tea tree oil to 2.5% sodium hypochlorite (NaOCl) solution as an intracanal irrigant for the removal of the smear layer.

Methodology Forty extracted, single-rooted, mature, permanent, human teeth were allocated at random into one of three experimental groups of ten teeth and two control groups of five teeth. For each tooth, the pulp chamber was accessed and the canal prepared using K-type files and Gates-Glidden burs, using a step-back technique; the apical stop was prepared to a size 30. Each canal was subsequently irrigated with one of the following solutions: distilled water (as a negative control), 2.5% NaOCl + 17% ethylenediamine tetraacetic acid (EDTA) (as a positive control), chamomile or tea tree oil or 2.5% NaOCl. Each tooth was split longitudinally and prepared for examination by scanning electron microscopy (SEM). The quantity of smear layer remaining on the three levels of each canal (coronal, middle and apical) was examined using magnifications of 2000 and 5000×. The data were analysed using nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests.

Results The most effective removal of smear layer occurred with the use of NaOCl with a final rinse of 17% EDTA (negative control) followed by the use of a chamomile extract. Chamomile extract was found to be significantly more effective than distilled water and tea tree oil ($P < 0.008$).The use of a 2.5% NaOCl solution alone, without EDTA and that of tea tree oil, was found to have only minor effects. There was no statistical difference between distilled water, 2.5% NaOCl and tea tree oil.

Conclusions The efficacy of chamomile to remove smear layer was superior to NaOCl alone but less than NaOCl combined with EDTA.

Keywords: chamomile extract, irrigation solution, smear layer, sodium hypochlorite, tea tree oil.

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Introduction

When the dental pulp undergoes pathological changes caused by trauma or caries, microorganisms enter the pulp chamber and invade anatomic irregularities of the root canal system. Successful root canal treatment is dependent on the removal of these microorganisms through chemo-mechanical instrumentation in which the shaping phase enhances the action of intra-canal medicaments and permits better adaptation of filling materials (Peters & Barbakow 2000).

McComb & Smith (1975) were the first to describe the smear layer in instrumented root canals through

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the use of scanning electron microscopy (SEM). The smear layer consists of organic and inorganic substances (McComb & Smith 1975). The presence of the smear layer has been postulated to be an avenue for leakage (Karagoz-Kucukay & Bayirli 1994), as well as to provide a substrate for bacterial growth and ingress (Ando & Hoshino 1990). While root canal shaping can be predictably and efficiently performed with instrumentation, effective cleaning of the entire root canal system with a suitable irrigant remains a challenge (Torabinejad *et al.* 2002). Many irrigants, such as sodium hypochlorite (NaOCl) have been used in root canal treatment (Torabinejad *et al.* 2002). NaOCl has excellent properties of tissue dissolution and antimicrobial activity that make it the irrigant of choice for the treatment of teeth with necrotic pulp, even though it has several undesirable characteristics such as toxicity, allergic potential, and a disagreeable smell and taste (Segura *et al.* 1999). In addition to its antimicrobial action and low toxicity, an ideal irrigant should have the capacity to clean the walls of the root canal and remove the smear layer.

Consumption of preparations from medicinal plants has increased over the last few decades. Two herbal preparations; chamomile (*Marticaria recutita*) hydroalcoholic extract and tea tree oil (*Melaleuca alternifolia*) have been studied for a range of properties such as antimicrobial activity (Martines *et al.* 2001, Hammer *et al.* 2003), biocompatibility, anti-inflammatory effects (Tubaro *et al.* 1984, Soukoulis & Hirsch 2004) and antioxidant properties (Safayhi *et al.* 1994).

Chamomile has been used for centuries as a medicinal plant mostly for its anti-inflammatory, analgesic, antimicrobial, antispasmic and sedative properties. German chamomile, in particular, is the most commonly used variety. It is an annual plant, native to Europe and Western Asia, and is used in parts of the world as a table tea. It is in fact the flower of the chamomile plant, which contains a wide variety of active chemical components, which are thought to be responsible for many of its medicinal applications (Tubaro *et al.* 1984). The tea was believed to make an excellent wash for sore and weak eyes and also for the treatment of open sores and wounds (Ruszynska *et al.* 1986). Chamomile was also found to be effective when used as a mouthwash to treat irritations and minor infections of the mouth and gingivae (Fidler *et al.* 1996) and is also used in some toothpastes (Ruszynska *et al.* 1986).

Melaleuca alternifolia, or Australian tea tree oil as it is more commonly known, is a native Australian plant with many properties such as being an antiseptic, an antifungal agent and a mild solvent (Hammer *et al.* 2003). Tea tree oil's major active component is terpinen-4-ol (typically 30–40%). This compound is responsible for its antibacterial and antifungal properties (Hammer *et al.* 2003).

The purpose of this study was to compare the *ex vivo* capacity of hydroalcoholic extract of chamomile and tea tree oil to 2.5% NaOCl, on the removal of the smear layer using SEM.

Materials and methods

A total of 40 maxillary and mandibular, single-rooted, noncarious, extracted human teeth with fully developed apices were included in this study. The teeth selected ranged from 21 to 25 mm in length and had intact clinical crowns. Teeth with coronal restorations or root filling were excluded. The teeth were randomly divided into three experimental groups of 10 teeth each and two control groups of five teeth each. After conventional access preparation for each tooth, a 10 or 15 K-type file (VDW, Munich, Germany) was introduced into the root canal until it could be seen at the apical foramen; it was then withdrawn to be within the apical foramen. The root canals were mechanically prepared using a stepback technique with a K-type file and Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) to size 30 at the working length. During instrumentation 2 mL of the selected irrigant was used for at least 10 s after each file. The irrigant was delivered using a 30-gauge endodontic tipped needle placed as far apically as possible into the canal, without binding. Each of the five groups (A–E) were treated with one of the following solutions: group A, sterile distilled water (negative control); group B, 2.5% NaOCl + 17% ethylenediamine tetraacetic acid (EDTA) (positive control); group C, hydroalcoholic extract of German chamomile (ACECR-Institute of Medicinal Plant Research, Tehran, Iran); group D, tea tree oil (Felton Grimwade & Bickford Pty Ltd, Oakleigh, South Victoria, Australia); group E: 2.5% NaOCl (Milton solution, Canberra, Australia).

After preparation of the root canal, a 10 mL final flush with the appropriate irrigant was carried out for 2 min. In groups A, C, D and E the final flushes were as for the intra-instrumentation irrigants, however in group B (the positive control) NaOCl was used during preparation of the root canal and EDTA was used as a final flush.

Longitudinal grooves were made on the buccal and palatal surfaces of the root segments without penetrating into the canal. The roots were then split

into two halves with a chisel. The split halves were stored in a 2.5% glutaraldehyde solution. After fixation, the samples were dehydrated in ethanol series (70%, 90%, 95% and twice at 100%) and then critical point dried using the dry ice method (BAL-TEC AG, Balzers, Lichtenstein, Lichtenstein). Each specimen was mounted on an aluminium stub and sputter coated with a ~20 nm layer of gold, to render a conductive surface. The specimens were examined using a SEM JEOL 6400 (JEOL, Tokyo, Japan) and Cambridge S360 (Cambridge, UK) SEM at magnifications of 2000 and 5000×. The specimens were blind coded. Analysis of the SEM images was performed by two investigators who scored the presence of smear layer on the surface of the root canal in the coronal, middle and apical portion of each canal based on the criteria described by Hülsmann *et al.* (2002) outlined below:

Score 1: dentinal tubules completely opened;

Score 2: more than 50% of dentinal tubules opened;

Score 3: less than 50% of dentinal tubules opened; and

Score 4: nearly all of the dentinal tubules covered with smear layer.

The data were further analysed using statistically based, nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests.

Results

In Group A, in which distilled water (negative control) was used as the irrigant, the dentinal walls were completely covered by smear layer. Even at high magnification no dentinal tubules could be detected (Fig. 1a). No smear layer was noted on the surface of the samples irrigated with 2.5% NaOCl and a final rinse of EDTA (positive control; group B) (Fig. 1b). The specimens irrigated with 2.5% NaOCl (group E) had moderate to heavy smear layer, which covered the apertures of the dentinal tubules, especially in the apical third. Occasionally, the location of a few tubules was apparent (Fig. 1c). A moderate to thin smear layer was seen in specimens treated with chamomile extract (group C), especially in the middle and coronal sections (Fig. 2a) but in the apical section there was more smear layer (moderate to heavy). In three cases from this group, however, smear plugs could be observed in the apertures of the dentinal tubules. Group D, which was treated with tea tree oil, had substantial smear layer in all sections (Fig. 2b).

Chamomile extract was found to be significantly more effective at smear layer removal than distilled water and tea tree oil ($P < 0.008$). Only in the apical section of the root canal, there was no statistical difference between chamomile and 2.5% NaOCl (Table 1). Statistical analysis revealed that distilled

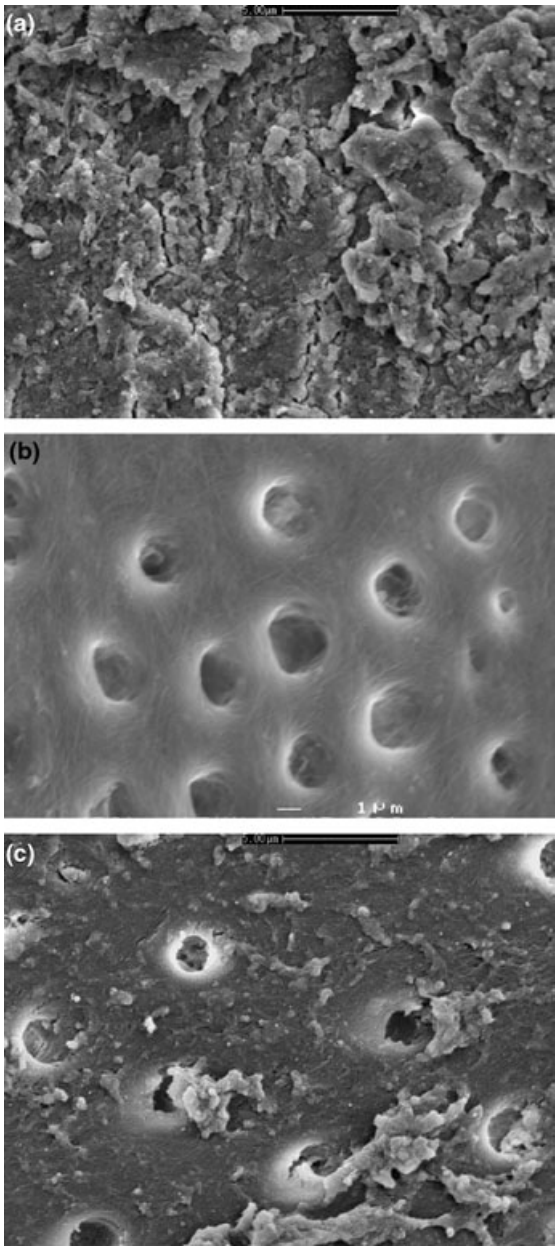

Figure 1 (a) Smear layer on the coronal surface of a root canal irrigated with distilled water. (b) Irrigation with 2.5% sodium hypochlorite (NaOCl) and 17% EDTA resulted in complete removal of the smear layer. (c) Smear layer on the surface of a root canal irrigated with 2.5% NaOCl (original magnification 5000×).

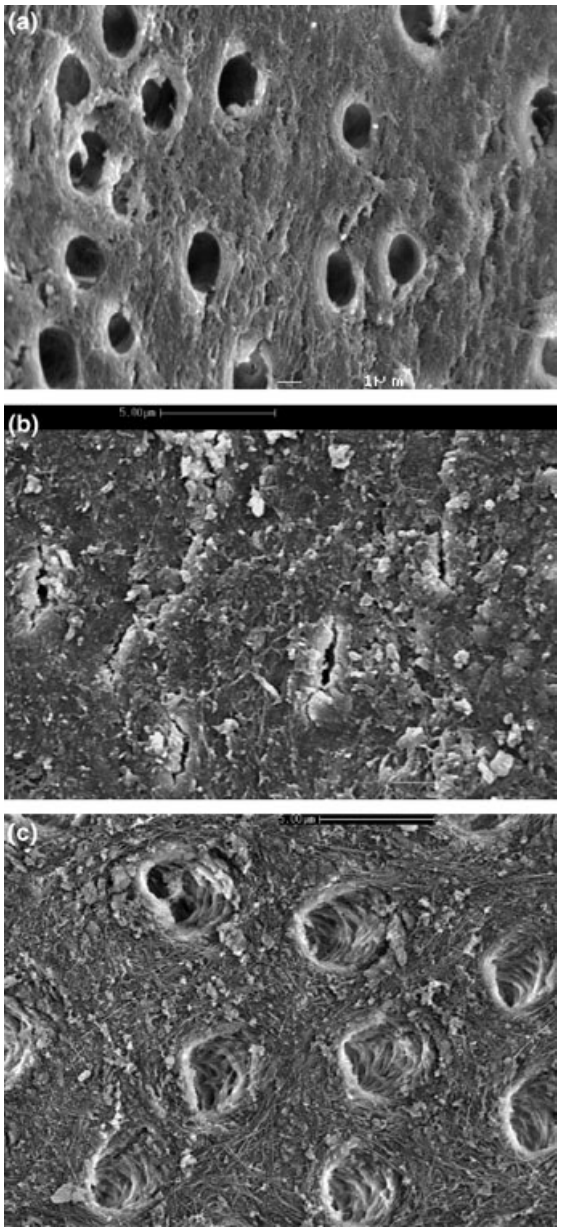

Figure 2 (a) Irrigation with chamomile extract resulted in moderate removal of the smear layer. (b) Heavy smear layer on the surface of a root canal irrigated with tea tree oil. (c) Moderate erosion of the dentinal tubules in the coronal portion of a root canal irrigated with 2.5% sodium hypochlorite and 17% EDTA (original magnification 5000×).

water and tea tree oil were not effective in removing the smear layer and the difference between them was not statistically significant. There was a significant difference between the NaOCl + EDTA group (negative control) and all other groups at different levels of the root ($P < 0.0008$).

Table 1 Comparison of different irrigation regimens using Mann–Whitney *U*-test

Irrigant	Apical third	Middle third	Coronal third
Sterile distilled water (A)			
Chamomile (C)	0.004	0.001	0
Tea tree oil (D)	0.604	0.836	1
NaOCl (E) 2.5%	0.075	0.033	0.033
NaOCl + EDTA (B) 2.5%	0	0	0
NaOCl (E) 2.5%			
Chamomile (C)	0.038	0.001	0.001
Tea tree oil (D)	0.007	0.039	0.01
NaOCl + EDTA (B) 2.5%	0.001	0.001	0.001
Tea tree oil (D)			
Chamomile (C)	0	0	0
NaOCl + EDTA (B) 2.5%	0	0.001	0.001
NAOCl + EDTA (B)			
Chamomile (C)	0.001	0.005	0

After Bonferroni correction, $P < 0.008$ was considered statistically significant.

Discussion

Endodontic instrumentation produces a smear layer that covers the root canal surfaces. This layer harbours microorganisms, infects dentinal tubules, impedes penetration or diffusion of antibacterial irrigants and medicaments into the dentinal tubules and compromises the seal between the filling materials and the dentinal wall. Because of its potential contamination and adverse effects on the outcome of root canal treatment, smear layer removal is recommended (Yamada *et al.* 1983).

In this study the entire canal length was utilized to simulate the clinical situation and to test the efficacy of the solutions in all segments of the root canal system. SEM was used to assess the effectiveness of various irrigants in removing the smear layer. Chamomile extract showed better cleaning in the coronal and middle thirds compared with 2.5% NaOCl. The results showed no significant difference in the ability of distilled water, tea tree oil and 2.5% NaOCl to remove the smear layer.

In line with other studies (Yamada *et al.* 1983, Baumgartner & Mader 1987) the results from Group B indicated that irrigation with 2.5% NaOCl during instrumentation with final flush of 17% EDTA for 2 min was significantly more effective in removing the smear layer compared with distilled water. This result concurs with result of other authors who have reported

that physio-chemical action of NaOCl is important in removal of organic residue, with EDTA acting mainly on the inorganic residue (Yamada *et al.* 1983, Baumgartner & Mader 1987). Small areas of erosion were noted in the coronal and middle third of the root canal walls (Fig. 2c). This finding is in agreement with other studies (Yamada *et al.* 1983, Baumgartner & Mader 1987), which reported a correlation between the erosive results of EDTA and the length of dentine exposure to this solution. Based on these studies and the present results, it appears that EDTA is destructive in the coronal and middle thirds of root canals if in contact with the root dentine for more than 1 min (Goldman *et al.* 1981). The results of the present study also demonstrated that conventional irrigation with 2.5% NaOCl alone (Group E) was comparatively ineffective in achieving thorough removal of the smear layer. This corroborates with earlier studies (McComb & Smith 1975, Baumgartner & Mader 1987).

In order to avoid the undesirable effects of NaOCl, two medicinal plant extracts which might disinfect the root canal system with less toxicity were selected for the study. Chamomile extract demonstrated better cleaning in the coronal and middle thirds. Small areas of erosion were seen in two specimens in this group. Few herbs have achieved such a reputation for use as chamomile. The chemical analysis of German chamomile has revealed its compounds to chamazolene, alpha-bisabolol and acids such as, capric acid, caprylic acid, chlorogenic acid, o-caumaric acid, p-caumaric-acid, dihydroxybenzoic acid and other components (Avallone *et al.* 2000). It would appear that the cleaning effect of chamomile in this study may be related to these acid components. The results of this study were limited to the physical changes to the root canal wall and did not evaluate *in vivo* effects and its possible effect on restorative materials.

In contrast to chamomile extract, tea tree oil did not clean the canal walls as effectively as distilled water and NaOCl. It was thought that a material with less surface tension might be more effective in removal of the smear layer (Goldman *et al.* 1981). The poorer results for tea tree oil might be related to its high surface tension. It might therefore be beneficial to change the extract of tea tree to a non-oily version. It might also be useful to test an aqueous extract or an emulsion of tea tree oil with less surface tension.

The cleaning effect of all the irrigants was more pronounced in the coronal and middle thirds than in the apical parts of the root canals. This finding concurs with other studies (McComb & Smith 1975, Yamada *et al.* 1983, Torabinejad *et al.* 2003). The smaller diameter of the root canal and the consequent decrease in the flow of the irrigant is the most probable explanation. Modification of the technique used for the introduction of the irrigant solution, for example by using perforated needles (Yamada *et al.* 1983), may have resulted in cleaner canals.

Conclusion

The most effective removal of smear layer occurred with the use of NaOCl with a final rinse of 17% EDTA (positive control) followed by the use of chamomile extract. A 2.5% solution of NaOCl alone did not produce satisfactory results. A less effective cleaning effect was found in groups in which either distilled water or tea tree oil was used as irrigants. The cervical and middle thirds were generally cleaner than the apical third. Further research is needed to determine the effects of chamomile extract on restorative materials and the effects of these extracts on the long-term prognosis of endodontic treatment.

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