EFFECT OF DIFFERENT ESSENTIAL OILS ON HUMAN DENTINE STRUCTURE

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Abstract

This paper revealed the effect of *Oregano*, *Rosmarinus* and *Myrtus* essential oils on the dentine structure stability for the improvement of collagen resistance against enzymatic activity. Several techniques were used: Fourier Transform Infrared Spectroscopy (FTIR), enzymatic degradation and Scanning Electron Microscopy (SEM). Results showed that all studied oils acted as protective films on the dentine after digestion of collagenase, even if the *Oregano* or *Myrtus* essential oils caused a demineralization process at the beginning. The oil treated slabs presented a significant lower mass loss than the non-treated slab after the action of the collagenase. This fact indicates a beneficial impact of these essential oils on the stability of the dentine.

Rezumat

Acest studiu evidenţiază efectul uleiurilor esenţiale de oregano, rozmarin şi mirt asupra stabilităţii structurii dentinare, în vederea îmbunătăţirii rezistenţei colagenice faţă de activitatea enzimatică. Tehnicile utilizate au fost: spectroscopia în infraroşu (FTIR), degradarea enzimatică şi microscopia electronică de baleiaj (SEM).Rezultatele au arătat că după acţiunea colagenazei, uleiurile formează o peliculă protectoare la nivelul dentinei deşi, iniţial, uleiurile de oregano şi mirt au determinat un proces de demineralizare. După acţiunea colagenazei, secţiunile dentinare tratate cu uleiuri esenţiale au prezentat o pierdere de masă mult mai mică faţă de cele netratate. Acest studiu indică efectul benefic al uleiurilor asupra stabilităţii dentinare.

Keywords: human dentine, essential oils, enzymatic degradation, SEM

Introduction

Dental caries and periodontal diseases are qualified as the most important in oral health problems, because they also affect the general health. Essential oils (EOs) are natural medicines used, with implication in dental treatment. In the last years, medicinal plants have become the subject of numerous research studies, proving their potential benefits to humans by different traditional, complementary or alternative treatment [2]. Plant volatile oils called essential oils represent a safe source of biologically active compounds. EOs are known for many purposes due to their antioxidant effects as well as their anti-inflammatory properties or antimicrobial activity [10-12] and in inhibiting plaque [3, 7, 8], but their applications are related to the main components of EOs. These oils are natural materials and complex systems that contain a variety of volatile molecules like terpenes and terpenoids, phenol-derived aromatic components and aliphatic components depending on the type of extraction from different parts of the aromatic plants. EOs can be used in different ways, and also can

be diluted before application, with no disadvantage for the therapeutic result. A type of application consists in placing the EOs on the teeth or on other parts of the body. Factors like the type of the volatile plant oil, plant part used and also teeth type and source will influence the chemical composition of EO and its effect on the dentine structure [4]. Evaluation of medicinal plants for antimicrobial and antioxidant activity is very important for finding new therapeutic materials. *Oregano* essential oil (OEO) is widely accepted as a traditional herb with the strongest antibacterial and antioxidant effect [6], and important antifungal properties due to the major components, carvacrol and thymol. Some studies demonstrated the antibacterial and antimutagenic properties of *Rosmarinus* essential oil (REO) [9] and the antioxidant, antimicrobial, antifungal activities and analgesic effects of *Myrtus* essential oil (MEO) extracted from leaves.

The aim of this study was to characterize the effect of three essential oils on the dentine structure stability, in an attempt to use EO as natural product for the improvement of collagen resistance against enzymatic activity.

Materials and Methods

Essential oils. Three essential oils such as *Origanum syriacum* L., *Rosmarinus officinalis CT 1,8 Cineol* and *Myrtus communis* L. were used for human dentine treatment. These EOs were selected based on their properties already observed in literature in traditional treatment [2, 8, 10]. All the plants used were harvested during the full bloom period and after were dried at room temperature. EOs were extracted by steam distillation from leaves of plants using special equipment developed by Mahan Cosmetics in Hatay. EO form of *Origanum syriacum* L. consists of natural phytochemical compounds, mostly phenols which have antioxidant effects. The main components are: thymol, carvacrol, γ-terpinene, cymol, α-terpinene. *Rosmarinus officinalis CT 1,8 Cineol* includes α-pinene, borneol, bornyl acetate β-pinene, 1,8-cineole, camphor, camphene and limonene. *Myrtus communis* L., usually known for its disinfectant, antiseptic and hypoglycaemic actions contains eucalyptol, δ-3-carene and α-terpineol.

For dentine sample preparation, 3 human upper molars were collected from different patients after their informed consent was taken, in a dental practice. After the enamel was entirely removed from all the surfaces, 2 consecutive transversal sections were obtained from each extracted tooth. The dentine slabs were then split into 3 groups. A non-treated EO and non-immersed in CLG slab was used as control sample (A0). All the sections were then sterilized at 254 nm with a Vilber-Lourmat equipment and demineralised in 0.5 M EDTA solution (pH = 7.4) for 15 min. After a washing protocol (3 times in deionized water) and a drying protocol (desiccator; 24 h; 36°C), gravimetric measurements were made for each section before and after the EO treatment with the specified natural extracts (3.75%) for 2 h (optimum immersion period).

Table I
Dentine slabs

Non-treated slabs	EO treated slabs		
immersed in CLG	type of EO	immersed in CLG	non-immersed in CLG
A1	OEO	A2	A3
B1	MEO	B2	B3
C1	REO	C2	C3

FTIR spectroscopy was used to identify specific functional groups in human dentine and in EOs and to evaluate the effect of EOs treatment on the dentine stability. FTIR spectra were recorded with **a** Perkin-Elmer spectrum 100 FTIR equipped with Attenuated Total Reflection (ATR) device. For data acquisition, the hard solid samples were analysed using diamond as hard ATR crystal material. In all samples, IR spectra were analysed in 4000 - 600 cm^{-1} domain by 32 scans, with a resolution of 4 cm^{-1}.

Enzymatic degradation test was done using *Clostridium histolyticum* collagenase from Sigma-Aldrich (USA). The prepared dentine slabs were hydrated for 1 h, immersed in collagenase solution (CLG) (0.1%) for 12 h, and incubated at 36°C under shaking walls. The dentine sections were washed, dried and gravimetric measured. The percentage dentine mass remained after the digestion of collagenase was calculated according to Equation 1:

$$\% \text{ dentine mass remained} = (m_2 \times 100)/m_1 \quad (1),$$

where m_1 and m_2 are dentine mass before and after collagenase treatment.

Scanning Electron Microscopy analysis. To observe the dentine surface morphology of the treated and non-treated human dentine slabs, all the samples were examined by scanning electron microscopy with a Hitachi S2600N SEM with an acceleration voltage of 15 kV.

Results and Discussion

Fourier transformation infrared (FTIR) spectra
The IR spectra of EO and human dentine slab as control sample were compared with that of the EO treated dentine slabs. Also, the treated dentine slabs were immersed in collagenase solution and analysed in order to evaluate the effect of the EO on the dentine structure stability. The IR spectra of EOs are presented in Figure 1.
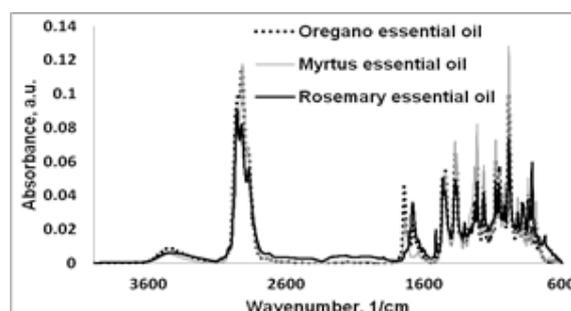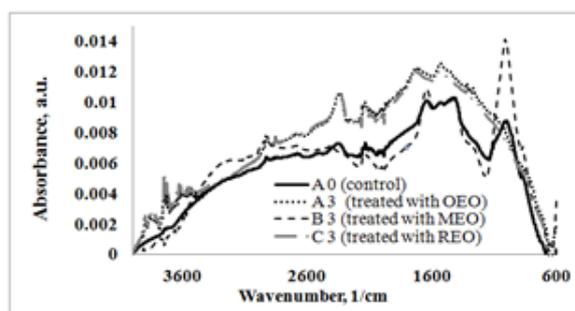

Figure 1.
IR spectra of EOs

A large number of the absorption bands [5] are evidenced; the most important are being as below. The bands in the region of 3452 - 3432 cm^{-1} are attributed to the stretching vibration of the phenolic hydroxyl groups (ν_{O-H}). The intense bands at 2960 - 2920 cm^{-1} and 2878 cm^{-1} correspond to ν_{asCH} and

ν_{sCH} in $-CH_2$ and $-CH_3$ groups, respectively. Peaks around 1740 - 1644 cm^{-1} correspond to $\nu_{C=O}$. ν_{C-C} from aromatic ring are registered at 1515 cm^{-1}. The bands at 1375 - 1359 cm^{-1} are due to the bending of $-CH_3$ group in $CH_3(CO)$. $\nu_{as(C-O-C)}$ and $\nu_{s\ (C-O-C)}$ are registered in 1272 - 1053 cm^{-1} domain. The bands obtained in the region 1017 - 650 cm^{-1} could be assigned to out – of – plane CH wagging vibration ($\omega_{(C-H)}$) in the aromatic ring.

Figure 2 shows the IR spectra of A3, B3, C3 and that of A0. Dentine composition consists in inorganic and organic materials and a part of water, the main components being hydroxyapatite, and Type I collagen. The IR spectrum of A0 contains specific bands as: $\nu_{C=O}$ of Amide I, δ_{N-H} and ν_{C-N} of Amide II from collagen appeared at 1638 cm^{-1} and 1530 cm^{-1}, respectively; other typical bands for dentine proteins are $-CH_2-$ bending vibrations at 1434 cm^{-1} and 1416 cm^{-1}; from the mineral part [1], the orthophosphate group is registered by the most intense absorption band at 1003 cm^{-1} (PO_4^{3-}); CO_3^{2-} vibration appeared at 874 cm^{-1}.
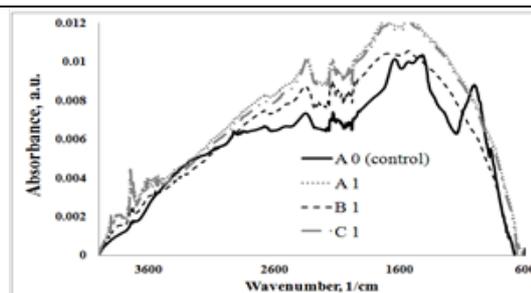

Figure 2.
IR spectra of EOs treated slabs

Comparing the spectra of A3, B3, C3 with that of A0, an increase in the intensity of the band around 1012 cm^{-1} is registered for B3 and it could be assigned to out – of – plane CH wagging vibration ($\omega_{(C-H)}$) in aromatic ring, specific for REO and also to PO_4^{3-} group from the mineral part of dentine. In B3 sample, CO_3^{2-} is recorded at 869 cm^{-1}. For A3 and C3, the bands corresponding to PO_4^{3-} and CO_3^{2-} groups were registered but with a very small intensity, which means that demineralization process occurred when dentine and OEO or MEO were in contact.

As seen from Figure 3, an important decrease in the intensity of the absorption bands corresponding to PO_4^{3-} and CO_3^{2-} in 1003 cm^{-1} - 874 cm^{-1} domain indicates a significant decrease in mineral components in the non-treated dentine slabs after digestion of the collagenase (A1, B1, C1). Loss of mineral components indicates that demineralization took place in all non-treated dentine slabs immersed in CLG.

Figure 3.
IR spectra of non-treated slabs immersed in CLG

In order to put in evidence the effect of different EOs on human dentine structure, Figure 4 presents the IR spectrum of A0 and those of EO treated dentine slabs after digestion of the collagenase (A2, B2, C2). While the treatment with REO did not affect the mineral phase of dentine, but the OEO and MEO caused decreases in mineral composition (Figure 2), however, after immersion in CLG, all treated slabs show the same shape of spectra and the same demineralization (Figure 4). Results lead to the conclusion that after REO treatment, the dentine structure is not affected, but demineralization occurred after immersion of it in CLG. The OEO and MEO dentine treatment caused an important demineralization process, but after immersion in CLG the demineralization did not continue.
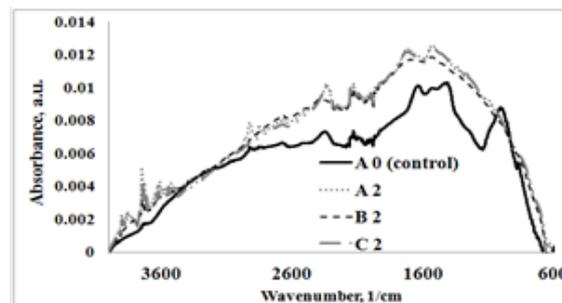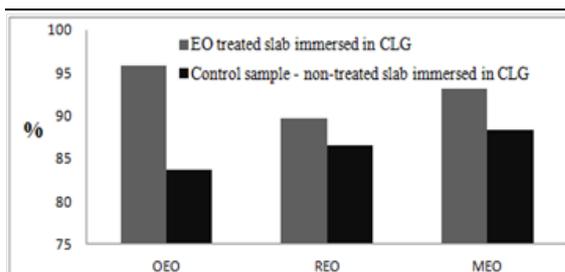

Figure 4.
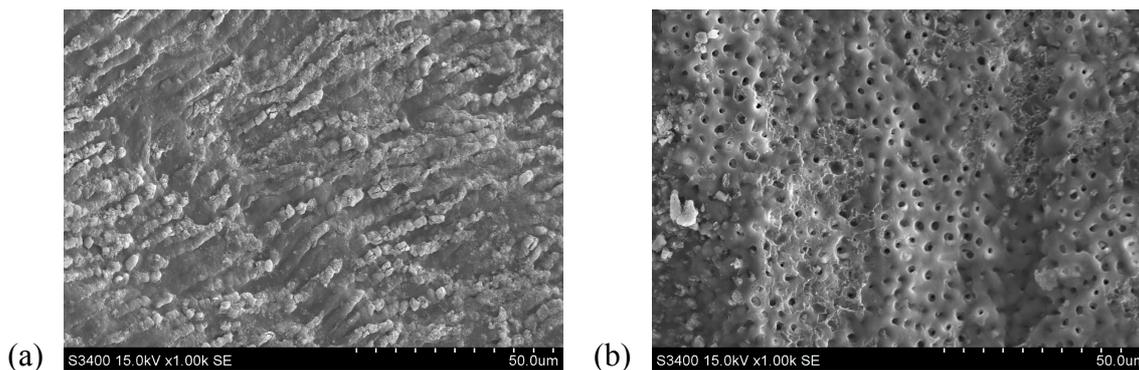IR spectra of treated slabs immersed in CLG

Enzymatic degradation
Figure 5 shows the results of the gravimetric measurements for the control samples and for the EO treated slabs. It can be observed the differences of dentine mass remained after the digestion for the control samples and for the EO treated samples. The treated slabs present a lower mass degradation than the control, fact that indicates the improvement of the dentine stability when treated with EO mentioned.

Figure 5.
Dentine mass remained for treated and non-treated slabs immersed in CLG

SEM analyse
Figures 6a and 6b show SEM images of non-treated and OEO treated slabs, both samples originate from the same tooth. The major difference between these images is that the OEO treated one presents orifices due to the action of the oil. These orifices could improve the durability of the adhesive that is applied in the dental practice after the demineralization since there is a much more surface for the adhesive material.

Figure 6.
SEM image of control sample (a) and OEO treated slab (b)

Conclusions

The REO treatment did not affect the mineral phase of dentine structure, but demineralization process took place after the digestion of the CLG. For OEO or MEO dentine treatment an important demineralization process occurred, but this process did not continue after the digestion of the collagenase. Even if the OEO or MEO caused an important demineralization process, after immersion in CLG it acts as a protective film so that the demineralization does not continue. The results show the beneficial impact of the studied EOs on the stability of the dentine surface, suggesting the possibility of the EOs application as natural product for dentine treatment by improvement the collagen resistance against enzymatic degradation.

Acknowledgement

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