IMPACT OF SOME ESSENTIAL OILS ON THE COLLAGENIC STABILITY OF THE HUMAN DENTINE

Filip IACOB[1], Gratiela Teodora TIHAN[2]*, Roxana Gabriela ZGARIAN[3]*, Georgeta VOICU[4], Ileana RAU[5]*

The filling failure appears at the marginal degradation between the filling material and the dentine, as a consequence of the weak structure of the dentine surface. This study proposes the treatment of the dentine with natural extracts. Different dentine slabs were treated with various essential oils (oregano, rosemary and myrtus), and tested under the action of collagenase. The results obtained showed that this treatment with the essential oils could improve the mechanical structure of the dentine.

Keywords: dentine, essential oils, oregano, rosemary, myrtus, collagenase

1. Introduction

In the current dental practices, the composite filling material represents the common method for coronal restauration. The standard procedure implies the application of an adhesive on the demineralized surface of the prepared cavity before the application of the composite resin.

The main cause of the filling material failure is represented by the marginal integrity degradation. This process appears between the surface of the cavity and the adhesive. Both surfaces, dentin/enamel and adhesive, form a hybrid layer. The marginal integrity degradation appears as a result of some reactions that take place at the dentine/adhesive hybrid layer level. The hybrid layer can be porous and be similar to a permeable membrane [1], allowing the removal of the unreacted monomers, water absorption, polymers enhancement, resin hydrolysis [2] and also allowing the enzymatic activity that causes the degradation of the fibrillar collagen type I, localized at the surface [3]. Thus, the two main causes that lead to the hybrid layer degradation are: the resin loss from the interfibrillar

[1] PhD student., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: iacob_filip@yahoo.com
[2] PhD Lecturer, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: gratielatihan@yahoo.com
[3] PhD Lecturer, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: zgirianroxana@yahoo.com
[4] Prof., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: getav2001@yahoo.co.uk
[5] Prof., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: ileana_brandusa@yahoo.com

spaces and the fibrillar collagen disorganization. Due to incomplete penetration of adhesive monomers into the interfacial layer, the collagen fibrils are not totally impregnated by resin as they were by hydroxyapatite mineral, making them predisposed to hydrolysis and enzymatic degradation.

The dentin includes an organic component that represents 20 % of its entire structure. The rest of 70 % is represented by the mineral component and 10 % by the fluid part. The organic part is formed by fibrillar collagen type I (90 %) and by noncollagenic proteins (10 %). The fibrillar collagen is the key element concerning the stability, tension, elasticity and the architecture of the collagenic fibers from the dentin surface. Its most important aspect is that it consists of a tridimensional reticular matrix that incorporates the mineral crystals. The fibrillar collagen type I is made of two α1 chains and one α2 chain. The intermolecular links between these chains represents the basis of the collagen's stability.

Therefore, the enhancement of the collagenic mechanical resistance of the dentin implies a strong intermolecular cohesion between the chains. Recent studies demonstrated that this cohesion can be improved due to the action of some cross-linkers [4]. Many studies were focused on obtaining such cross-linkers and the recent ones indicate a fenolic compound made by tannins, Proanthocianidin, as being the most effective in this direction [5-10]. Some of its advantages shown in these studies are: the improvement of the mechanical properties (elasticity modulus) from the hybrid layer and from the surface of the dentin, the enhancement of the synthesis of collagen, low toxicity or nule toxicity, the improvement of collagenic resistance against enzymatic activity, both *in vivo* and *in vitro* models [11-14].

The aim of this study was to test the impact of oregano, rosemary and myrtus essential oils on the stability of the surface of the dentin. These three natural extracts contain tannins in their structure and have never been used in a study concerning the surface of the dentin before. The main objective of this experiment is to find out the effect of the essential oils on the dentine after the collagenase action.

2. Materials and methods

Oregano essential oil contains phenols, which are natural phytochemical compounds with beneficial antioxidant effects. The two most abundant phenols are: thymol (a natural fungicide with antiseptic properties) and carvacrol (found to be effective against various bacterial infections, such as *candida albicans*, *staphylococcus*, *E. coli*, *campylobacter*, *salmonella*, *klebsiella*, the *aspergillus mold*, *giardia*, *pseudomonas*, and *listeria*). Other compounds present in the oregano oil are: terpenes, rosemarinic acid, naringin, beta-caryophyllin.

Rosemary essential oil (*Rosmarinus officinalis CT 1,8 Cineol*) is a steam-distilled oil taken from the flowering plant. The main chemical components of rosemary oil include α-pinene, borneol, β-pinene, camphor, bornyl acetate, camphene, 1,8-cineole, and limonene.

Myrtus essential oil is obtained from a very aromatic plant because of the high essential oil content in its leaf, flower and fruit glands. It is traditionally used as an antiseptic, disinfectant and hypoglycemic agent.

A number of three human premolars and three frontal teeth were sampled from different patients after their informed consent in a dental practice. The enamel was entirely removed from all the surfaces. Then two thin consecutive transversal sections were realized from each extracted tooth. All the sections were divided into 6 groups, each of them containing the two-paired sections (one sample being reference and the other one being treated with essential oil) from every premolar and frontal tooth.

Before the treatment with essential oils or collagenase solution, all these dentine slabs were firstly sterilized with a Vilber - Lourmat equipment. For this process, each side of the slab was maintained at 254 nm for 5 min. After that, the slabs were demineralized by immersing them for 15 s in 0.5 M EDTA solution (pH = 7.4). The dentine samples were washed 3 times in deionized water and dried in a desiccator for 24 h at 36 °C. Gravimetric measurements were realized for each dentine slab before and after treating them with the specified natural extracts (3.75 %) for 2 h (optimum immersion period). The samples were then hydrated for 1 h and immersed in collagenase solution (0.1 %) for 12 h, being incubated at 36 °C under shaking walls. The dentine sections were again washed, dried and gravimetric measured.

The surface morphology of the human dentine slabs was examined by scanning electron microscope technique (SEM) with a Hitachi S2600N apparatus. In order to increase the conduction electron beam, the slabs were coated with a layer of Ag before examination.

3. Results and discussion

Since the collagenase has an enzymatic degradation effect on the collagenic tissue, the comparison between its action on the essential oil treated samples and on the control ones may lead to a conclusion whether the essential oils can improve the stability of the surface of the dentin or not. Fig. 1 presents the influence of the essential oils treatment on the premolar dentine, while Fig. 2 shows the influence of these oils on the frontal dentine. It could be observed that in all cases the treatment with these oils has a beneficial influence. It should be underlined that the dentine degradation is depending on different factors which cannot always be quantified. Nevertheless, generally for the dentine without

treatment, up to 15 % of its weight is lost under the collagenase action. The treatment with essential oils appears to have as a consequence a significant decrease of the weight loss.

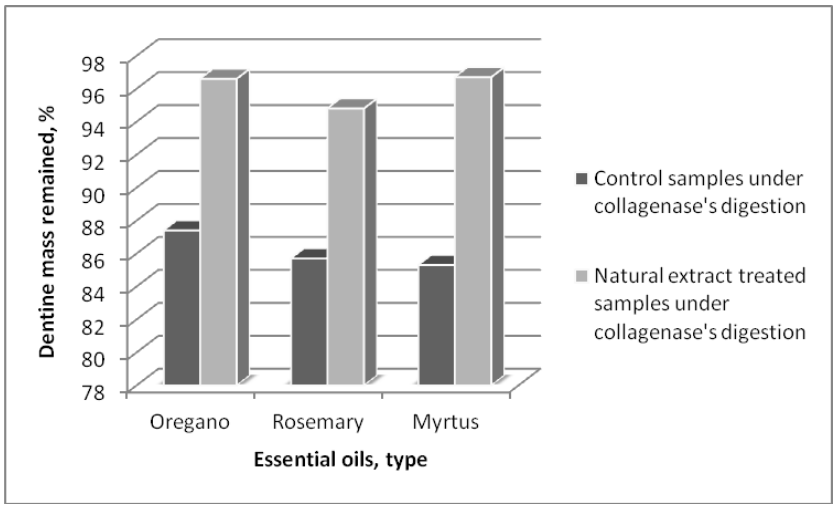

Fig. 1 – Percent premolar dentine mass remained after the digestion of collagenase

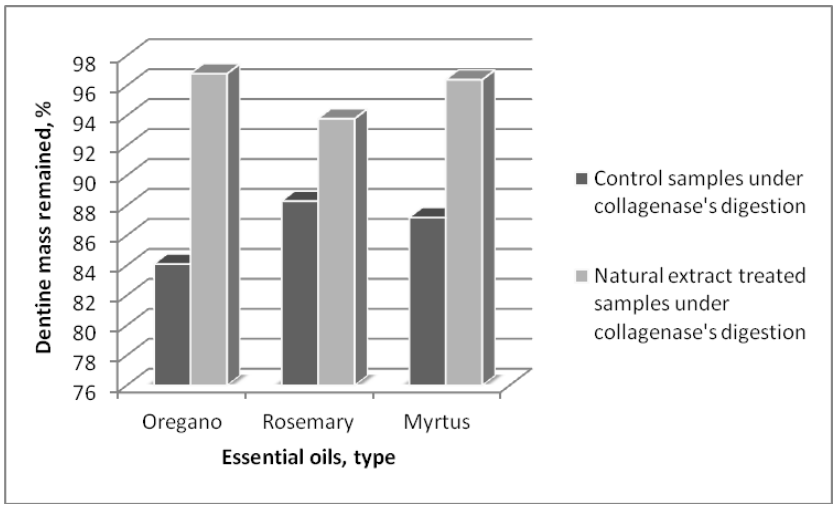

Fig. 2 - Percent frontal dentine mass remained after the digestion of collagenase

SEM images for the surfaces of the dentine slabs were registered before and after treating them with the natural extracts, in order to observe the differences produced by the essential oils on the collagen structure of the dentine.

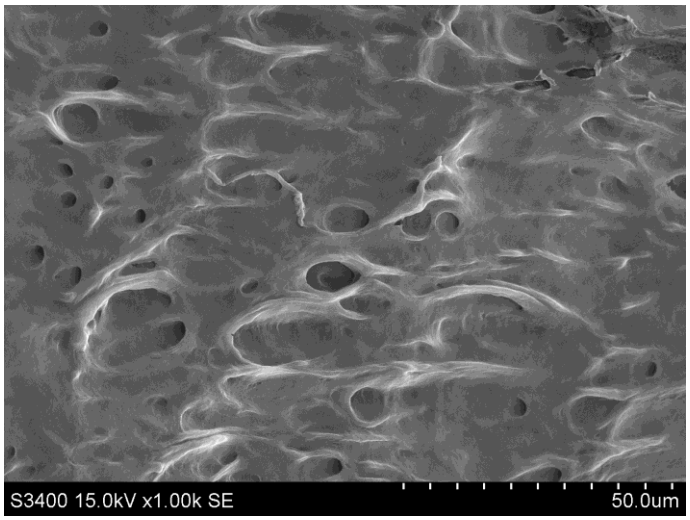

Fig. 3 – SEM image of a non-treated dentine section

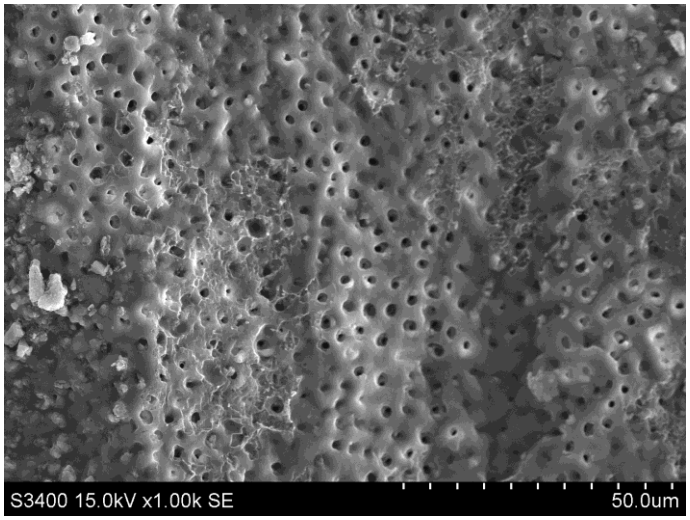

Fig. 4 – SEM image of an oregano essential oil treated dentine section

Fig. 3 represents a SEM image of a non-treated section, while Fig. 4 represents a SEM image of a dentine section treated with oregano essential oil. Similar images were registered for dentine slabs treated with the other essential oils. It could be seen that the oregano treated section presents deeper orifices at the surface than the non-treated section. This could be interpreted as the direct impact of the oregano essential oil on the dentine surface, leading to the conclusion that an improvement of the collagenic stability of the surface has been produced.

5. Conclusions

In the current study, the stabilizing properties of oregano, rosemary and myrtus essential oils for the collagenic dentine were demonstrated through gravimetric measurements and SEM imaging. This improvement of the stability of the surface could indicate a major step toward the durability of the adhesives on dentine, since a marginal degradation between the dentine and the adhesive has a lower risk to appear, due to a more stable cohesion of the two surfaces. The results presented here could be a starting point for future clinical trials, by including these natural extracts in the filling material dental procedures.

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