Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

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To cite this article: Filip Iacob, Graţiela Teodora Tihan, Roxana Gabriela Zgârian, Marinela Pauliuc, Ileana Rău & Cristian Pîrvu (2016) Preliminary studies concerning some natural extracts influence on dentin, Molecular Crystals and Liquid Crystals, 628:1, 110-114, DOI: 10.1080/15421406.2015.1137682

To link to this article: http://dx.doi.org/10.1080/15421406.2015.1137682

Published online: 13 May 2016.

MOL. CRYST. LIQ. CRYST.
2016, VOL. 628, 110–114
http://dx.doi.org/10.1080/15421406.2015.1137682

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Preliminary studies concerning some natural extracts influence on dentin

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ABSTRACT

In dentistry, one of the major problems is related to the adherence of the composite filling materials to the dentin. Presently, in order to enhance this adherence, different adhesive materials are used. The main cause for the filling failure is the marginal degradation appeared between the filling material and the dentin, due to the weak structure of the surface of the dentin. We propose here the treatment of the dentin with some natural extracts (enoxil and laurel essential oil) in order to improve its mechanical properties. The results showed that this treatment could improve the mechanical structure of the dentin.

KEYWORDS

Laurel essential oil; enoxil; collagenic dentin; AFM

Introduction

In the current dental practices, the most common method for coronal restoration is by using the composite filling material. This dental composite resins are usually composed of bisphenol A glycidyl methacrylate (Bis-GMA) and other dimethylacrylate monomers. The standard procedure for a coronal restoration implies the application of an adhesive on the demineralised surface of the cavity before the application of the composite resin.

The main cause of the filling material failure is the marginal integrity degradation. This process appears between the surface of the cavity (dentin/enamel) 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 dentin/adhesive hybrid layer level. The hybrid layer can be porous and similar to a permeable membrane [1], allowing removal of the unreacted monomers, water absorption, polymers enhancement, resin hydrolysis [2] and also allowing the enzymatic activity that causes a 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 the interfibrillar spaces and the fibrillar collagen disorganization. Due to incomplete penetration of adhesive monomers into the interfacial layer, the collagen fibrils are not completely 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

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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 $\alpha 1$ chains and one $\alpha 2$ 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 is 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 null 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 enoxil and laurel essential oil on the stability of the surface of the dentin. These two 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 the optimum time of action for these natural extracts on the collagenic structure in order to improve the mechanical properties.

Materials and methods

Enoxil and laurel essential oil were studied for their activity on the stability of the dentin. Enoxil represents a mixture of natural substances and it can be obtained by oxidizing the tannins collected from grape seed. Laurel essential oil is rich in oxides (cineol) and fenol-methyl-ethers (estragol, eugenol) and contains tannins, pectins, resins and lactones among others.

16 extracted human premolars were obtained from different patients after their informed consent in a dental practice. The enamel from the crowns was entirely removed from all the surfaces, occlusal and axial ones. Then were carefully realized 2 thin consecutive transversal sections from each premolar. All the slabs were divided into 16 groups, each of them containing the two-paired sections from every premolar.

All these dentin slabs were then sterilized (254 nm) and demineralised by immersing them for 15′ in 0.5 M Ethylenediaminetetraacetic acid (EDTA) solution (pH = 7.4). The dentin samples were washed 3 times in deionized water and then dried in a desiccators for 24 h (36°C). Gravimetric measurements were realized for each dentin slab before treating them with enoxil (3.75%) and laurel essential oil (3.75%) at relevant periods of time: 0 control, 10 s, 1 min, 30 min, 60 min, 120 min, 360 min, 720 min. The dentin sections were then again washed and dried for 24 h (25°C) until the gravimetric measurement was realized for each one.

After the optimum treating time in these two solutions was indentified, another test was realized in order to find out the impact of the collagen solution (0.1%) on the both treated and control samples. Therefore, another 4 extracted premolars were collected and prepared using the same procedure resulting 8 samples. Two consecutive transversal sections were obtained from each premolar (A1, A2, B1, B2, C1, C2, D1, D2). A1, B1, C1, D1 were utilized as control samples, while A2 and B2 were treated with enoxil solution and C2 and D2 with laurel essential oil. The same protocol of sterilization, demineralization, washing, drying and gravimetric measurements was respected, excepting the time immersion periods in essential oils that were

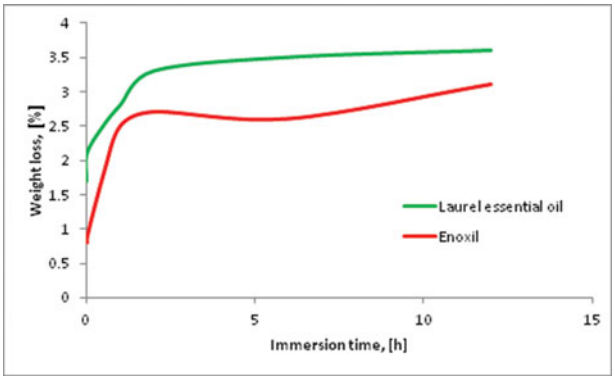

Figure 1. Percent weight loss after enoxil and laurel solutions treatment at relevant time periods

switched with the optimum time. The samples were hydrated for 1 h and immersed in collagenase solution (0.1%) for 12 h, being incubated at 36°C under shaking walls. The dentin sections were again washed, dried and gravimetric measured.

Atomic Force Microscopy (AFM) measurements were also realized in order to sustain the gravimetric measurements results.

Results

The dentin sections that were treated with enoxil and laurel essential oil solutions suffered a gradually weight loss. As it can be seen in Fig. 1, the degradation effect of the solutions starts immediately after the first relevant time periods of immersion, the highest peak of *treating time/weight loss* being 2 h. After this time interval, the solutions impact on the dentin collagen seems irrelevant for both solutions.

The optimum time being indentified, the aim of the next experiment was to study the collagenase solution impact on both treated and non-treated samples. 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 could lead to a conclusion whether the essential oils can improve the stability of the surface of the dentin or not. Fig. 2 clearly indicates that the weight loss in the case of the treated dentin sections is significantly lower than weight loss of the non-treated sections. This result could be interpreted as the positive effect of the enoxil and laurel essential oil solutions on the stability of the dentin surface.

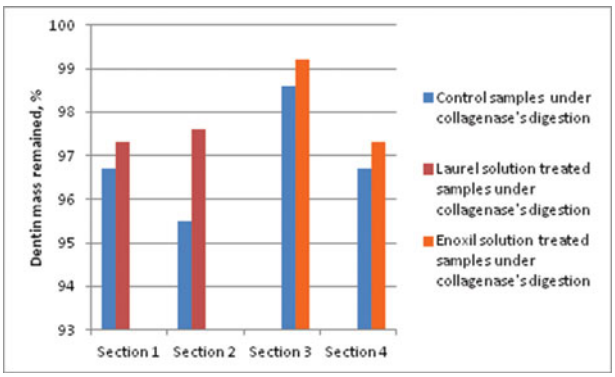

Figure 2. Percent dentine mass remained for the studied samples after the digestion of the collagenase

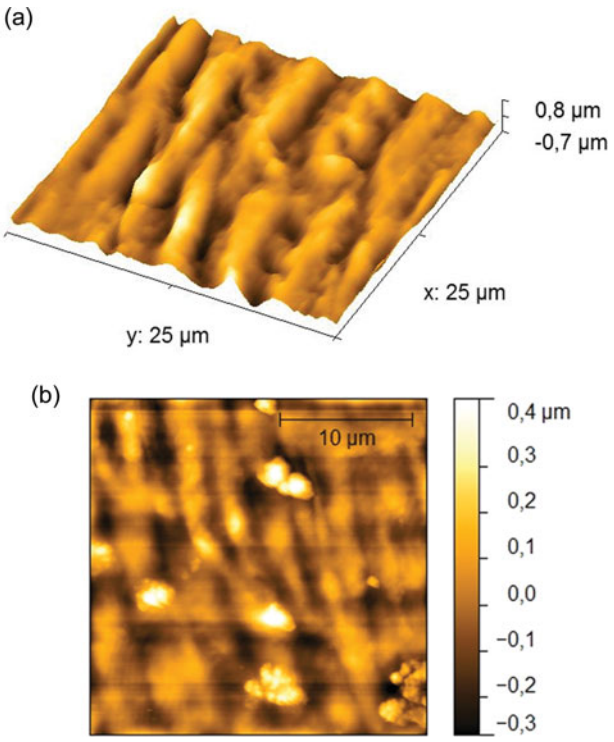

Figure 3. AFM images of the dentine surface before treating with enoxil (a) and after 2 h treatment with enoxil (b)

AFM images were realized on untreated sections and on 2 h enoxil treated sections (Fig. 3 a, b). The AFM images show a slightly increase of the diameter of the tubules orifices, fact that could explain the minor degradation effect of enoxil as resulted from the gravimetric measurements.

Conclusions

Dentin slabs were treated with some natural extracts in order to improve their surfaces for reducing the marginal degradation in time. In the current study, through gravimetric and AFM measurements, it was demonstrated that enoxil and laurel oil can lead to a beneficial action on collagenic dentin properties. This improvement of the surface stability could indicate that natural extracts are useful for dental treatment concerning the resistance in time of the adhesives on the dentin surface.

Acknowledgment

The work has been funded by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/137390.

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