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CHANGES ON DENTAL ENAMEL AFTER ACID ETCHING

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Abstract: Etch of enamel produces pores, where subsequently resin or adhesive system infiltrate. Silvestrone has established different morphological forms of etched enamel. Enamel surface, after being treated with phosphoric acid, has been demineralized in thickness of 5−10 μm, and that is enamel etched area. About 20 μm thick pores formed under the surface are the areas of qualitative pores. Under that, about 20 μm thick area of quantitative pores follows. Material consisted of human teeth with intact enamel extracted because of paradontophatia or orthodontic reasons. Untreated and treated teeth have been analysed with the application of AFM, type JSPM-5200 in *contact mode*, which means that physical contact between AFM sonda and enamel surface is constant force.

Keywords: etching, dental enamel, AFM analysis.

1. INTRODUCTION

In modern dentistry, one of the most important achievements has been development of the characteristics of adhesion of composite tissue on hard dental tissue, before all, on enamel and dentine. In 1955, Buonocore established the characteristic of adhesion on enamel [1], where he used phosphoric acid for erosion of enamel surface layer to extend the retentive surface and the free energy of surface. With the erosion of the enamel, the pores are created, in which, subsenquently, resin or adhesive system infiltrate. Silvestrone has established different morphological forms of etched enamel. Surface enamel, after infliction of phosphoric acid, is demineralised in thickness 5−10 μm and this is area of etched enamel. Under the surface, pores are formed, thick about 20 μm, and these are areas of qualitative pores. Under that area, an area of quantitative pores comes, about 20 μm thick.

Beside these, it is also possible to achieve adhesion successfully with the use of self-etch adhesions. Low-viscosity monomers infiltrate into the enamel surface, and create a hybrid layer as micromechanic retention, inside and around the enamel prisms. Considering that these structures may only be seen under enlargements (by electronic microscope), it is about nanoretention on the enamel surface. Finally, it ought to be observed that operation of self-etching adhesions on the tooth enamel is less agressive than phosphoric acid, where demineralisation of the surface is 1−2 μm.

Tooth enamel is constructed of billion of crystal carbonized hydroxiapatites [2-4] sorted into detached prisms which wind from the enamel-dental border to the tooth surface. Enamel prisms observed in diagonal section by using electronic microscope do not have an appearance of prism (grid), we observe them as key-hole shaped, 6-8 microne in diametre. In such an appearance, a wider part marked as 'head' differs from a narrower part marked as 'tail'. Each head is inserted between two tails. Crystals in the area of the head are arranged along vertical axis, marked as 'C axis', while at the periphery ('tail') the crystals are positioned at an angle of 30^o [5−7].

Enamel prisms are created by complex interaction of ecto-mesenchymal and ectodermal tissues and by coordination of the cells responsible for their ameloblast synthesis. Ameloblasts appear from enamel organ under inductive influence of ecto-mesenchymal cells that migrate into the area of stomatodeum. Ecto-mesenchymal cells lead to lace-like multiplying of ectodermal cells and creating a horse-shoe, marked as dental beam (dental lamina). In ten places of the beam, additional multiplying of the epithelium cells occurs in the appearance of a ball-like pile of cells marked as a tooth bud**.** Epithelium cells perform inductive influence on ecto-

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mesenchymal cells, they multiply and deepen a small ball, which gets form of a cap now. In this stadium of a bud, all formative structures differ from each other, which will become teeth tissue subsequently [8-12].

At mature enamel, mineral phase occupies about 87% of the total volume of enamel tissue; and makes more than 95% of material weight, from which only 5% belongs to organic materials and water (other biological mineralized tissues present about 20%). Porosities developed from the web of channels hold 3-5% of the volume. Ions and small molecules difundate through them and through the whole enamel liquid cover. This space exists between prisms, and also between crystals.

Morphological structures rich in proteins also join this web, as well as above mentioned retzius lines, enamel lamels, enamel bushes and enamel spindles. It has been considered that canalicular system performs a protective role for the following reasons: 1) it enables physiological remineralisation of enamel prisms during a life, and 2) space, liquids and proteins participate partly in big pressure amortisation which releases during chewing, and prevents fracture formation. Simultaneously, this canalicular system enables infiltration of acids, as well as bacteria, and assists development of caries and erosions [4−6,8,13].

Enamel surface is not even. Its structure is undulating, because in the place where retzius lines finish, their staircase overlapping occurs, and at that place, cavities marked as *perikymata* appear. In single places, especially at milk-teeth, several enamel microns without prismic organisation exist − *aprismatic enamel* [4,5]. Even though enamel owns expressive firmness, it is exceptionally fragile and glass-like, as though it may be inclined to crack. Despite, enamel sustains burden more than 1,000 N many times during the day. The entire enamel structure is formed to be accomodated to such burdens. Support of flexible dentine also contributes to it, as well as the structures like enamel bushes on enamel dentine boundary [10,12,14].

Enamel is in continuous dynamic communication with mouth cavity ecosystem. Process of demineralisation and remineralisation is always present and its balance provides enamel integrity. If outer agressive factors direct that balance towards demineralising activities, integrity of the crystal grid weakens, and the thickness and resistance of the enamel declines. Surpassing a certain border of enamel mechanic resistance leads to enamel fracture and cavity formations, and to the beginning of irreversible damage [8,13].

2. MATERIALS AND METHODS

The material consisted from human teeth with intact enamel, extracted due to paradontopathia or orthodontic reasons. The samples of the teeth enamel were 3mm x 2mm x 2mm sized and prepared according to the standard procedure. Untreated teeth deludged only in physiological solution made the first control group, and the experimental teeth were treated with 35% phosphoric acid. The analysis was performed with the application of AMF, type JSPM -5200 in *contact mode*, which means that the physical contact between AFM sonda and the tooth surface was constant force.

3. DISCUSSION AND RESULTS OF STUDY

Erosion of the enamel with acid causes a selective demineralisation which increases the free energy of the surface**.** Bonding to the enamel, adhesion [15] depends on the ability of resin to infiltrate into the space between crystal prisms [16] which leads to macro-mechanical retention. Infiltrated resin binds single hydroxylapatite crystals forming micro thorns [17] and creates a hybrid layer which realises mechanism of retention on nanolevel between the teeth and resin [18]. These micro thorns probably contribute to the adhesion more than macro thorns which enter the space between the enamel prisms [19].

Retentive abilities of the eroded enamel depend on chemical structure of the enamel mineral phase, type of acid and the time of erosion. Researches have proved that time variations of erosion of 15 to 90 seconds with 35-37% phosphoric acid do not influence the shear bond strength much.

During the time of erosion, the damages are greater, which is first manifested by engaging the complete enamel prisms, that occurs in the first 15 seconds. In the 15 and 30 seconds of the further course, destruction mostly extends engaging central regions of the prisms deeply [21].

Roughness is generally defined as a complex set of irregularities or convexities and cogs which give the surface view, and influence wetness, quality of the adhesion and illumination.

Even though it has been emphasized that micro-mechanic roughness is a basis of good cohesion between the erosed enamel and resin, the precise characteristics of the tooth enamel needed to realise such cohesion are still not known [22]. Also, the influence of the roughness to the strength of the cohesion has not been understood completely [23]. It

is assumed that higher roughness level provides more contact surface. That surface provides a contact with resin, and stronger cohesion as well [24].

So far, surface roughness on the microscopic level has not been examined in details, [25] where nano characterisation of the surface roughness may provide biophysical mechanisms on the enamel surface [26]. AFM with high lateral and vertical resolution enables the research of roughness on micro and macro levels without relevant involvement of macroscopic components such as undulating surace [27]. AFM micro trial does not require a preparation of the sample or endangering original surface. This way, it presents a direct way to detect and quantify the surface roughness experimentally [28].

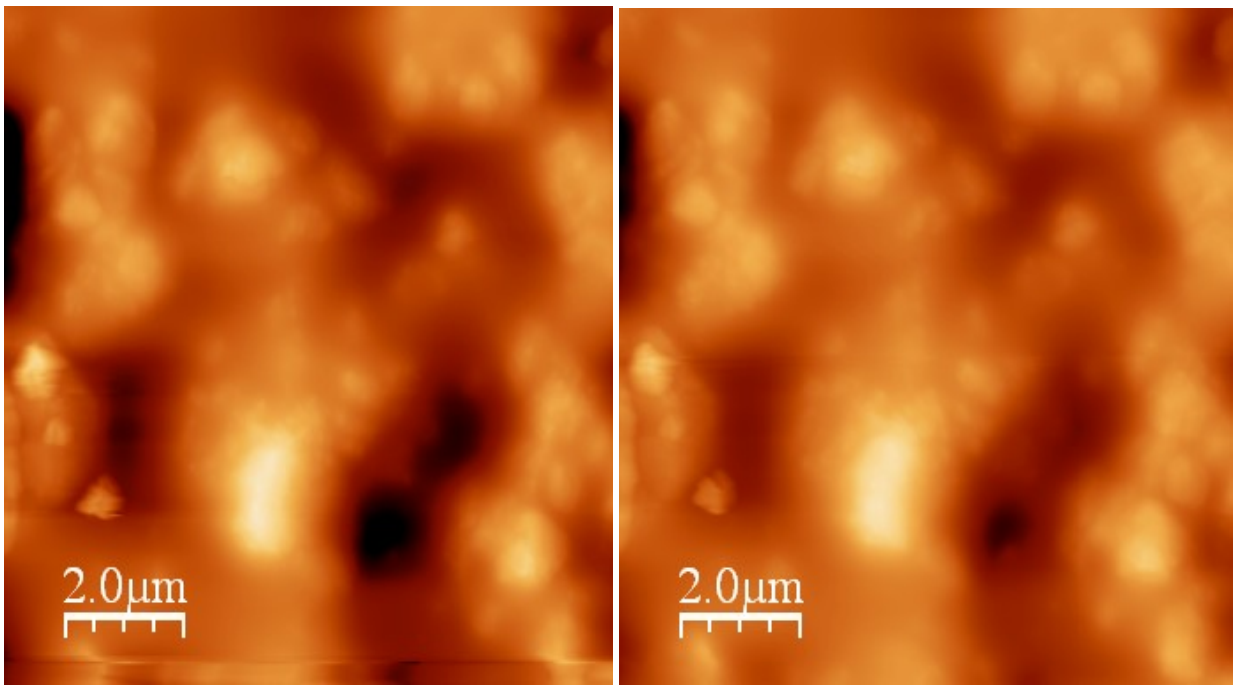

Figure 1. Stevia Group AFM images

Now we will provide overview of analysis which we carried out with Stevia Rebaudiana natural sweetener shown in photo 1 and provide comparison with presented results obtained with acid etching. Stevia group shows less expressed dents and overlay of prismatic indentations with a thicker coat of more organized structure. Here the minimum measured roughness is 3095, and maximum 3249 nm. The median is 3170, and the measured average roughness is 3165.28 nm, while standard deviation is 43.774 nm. AFM analysis of tooth surface treated with Stevia Rebaudiana in vitro shows pronounced surface roughness, and more organized crystal structure tending to create apatite which also indicates potential protective role especially in the phase of preparation of the existing damage. Interpretation of AFM findings only for explanation of changes to the enamel surface, especially in demineralization and re-mineralization mechanisms is not always sufficient and it is recommended to combine several methods, including also SEM analysis of crystal structure.

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ПРОМЈЕНЕ НА ГЛЕЂИ ЗУБА НАКОН ЈЕТКАЊА КИСЕЛИНОМ

Апстракт: Јеткањем глеђи стварају се поре у које касније продире смола или адхезивни систем. Силвестроне је установио различите морфолошке облике јеткане глеђи. Површина глеђи након наношења фосфорне киселине деминерализована је у дебљини 5−10 μm, а то је зона јеткане глеђи. Испод површине стварају се поре дебљине

око 20 μm, а то су зоне квалитативних пора, а испод те зоне слиједи зона квантитативних пора, дебљине око 20 μm. Материјал су сачињавали зуби човјека са интактном глеђи, екстраховани због парадонтопатија или из ортодонтских разлога. Нетретирани и трететирани зуби анализирани су помоћу АФМ-а типа m у *contact mode* што значи да је физички контакт између АФМ сонде и површине зуба константна сила.

Кључне ријечи: јеткање, глеђ зуба, АФМ анализа.