SEM Analysis of Hybrid Layer Adhesion in Substances Used for Dentinal Hypersensitivity

ATENA GALUSCAN[1], DANIELA JUMANCA[1]*, RAMONA AMINA POPOVICI[1], ANGELA CODRUTA PODARIU[1], LAURA CRISTINA RUSU[1], TEODORA STEFANESCU[2]

[1] University of Medicine and Pharmacy Victor Babes Timisoara, Romania, Faculty of Dentistry, Department of Preventive Dentistry, Community and Oral Health, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2] University of Oradea, Romania, Faculty of Medicine and Pharmacy

The objective of the present study was to determine the adhesion level of various desensitizing agents and a model for the hybrid layer on dental surfaces. Also, this study aims to establish if the composition and presentation of the desensitizing agent determine and influence the hybrid layer and its adhesion level. The study was conducted on 33 human teeth, third molars and premolars, extracted for orthodontic purposes. After extraction, each tooth was sectioned into two pieces thus obtaining 33 section pairs. One half of the samples were randomly divided in three groups and treated with three different desensitizing agents: Gluma Desensitizer (Hereus), Fluor Protector (Ivoclar) and Tooth Mouse (GC), available on the market and commonly used in dental offices, followed by SEM (scanning electron microscopy). The other 33 samples served as control group. The adhesion of desensitizing agents is achieved by formation of a hybrid layer on the dental surface and by creation of micro-tags which differ depending on the product's physical-chemical proprieties. Conclusions: With the aid of SEM imaging we were able to prove that Gluma Desensitizer generates the most homogenous hybrid layer on dental surfaces, followed by Fluor Protector and Tooth Mouse. The adhesive process is influenced by the presentation of the desensitizer (fluid or paste) and by the type of physico-chemical reaction leading to hybrid layer formation.

Key Words: dentinal hypersensivity, electronomicroscopy, desentizing agents, hybrid layer, adhesion

Modern lifestyle has led to various dental diseases, other than dental caries, which occur by loss or deterioration of the enamel. This enamel loss leads to Dentinal Hypersensitivity (DH). The etiology of this disease is represented by dental abrasion or erosion, occlusal trauma, pathological lip frenulum insertion. Attrition, abrasion or abfraction are traumatic lesions affecting dental enamel causing DH [1-3]. Once enamel is lost and cement or dentine exposed, these dental under-layers are subjected to massive erosion, as a consequence of lesser inorganic mineral content.

Clinically, DH is characterized by brief and profound pain due to a response to a thermal, tactile, volatile, osmotic or chemical stimulus acting on exposed dentine, and this pain cannot be associated to any dental defect [1]. DH is a disease with increased prevalence among patients (up to 74%). The profile of the patient suffering from DH varies with age between 20-50 years, with a peak of incidence between 30 and 40 years [3-6].

Anatomically, the dental pulp is integrally connected to dentine, physiologic and/or pathologic reactions in one of the tissues will also affect the other. Dentin consists of small canal like spaces, dentinal tubules where odontoblastic processes are taking place [5].

Brännström, stated and demonstrated that dentinal pain is due to a hydrodynamic mechanism. The hydrodynamic theory is the most frequently accepted for explaining the mechanism of DH. According to this theory, pain stimuli originating in the oral environment act on exposed dentine surfaces and cause a rapid movement of the fluid inside dentinal canaliculi. This movement stimulates mechanical receptors at dental pulp periphery leading to intense pain experienced during a brief period. It occurs after the protective cover of smear layer is removed, leading to exposure and opening of dentinal tubules. [7-9]

Consequently, the most appropriate treatment for DH is to obturate dentinal tubules by specific adhesion of certain substances to smooth dental surfaces.

Grossman formulated the requirements for an agent to achieve an ideal dentinal desensitization. These are: rapid long term action, immediate improvement, no pulp irritation, painless, easy to apply, no dental dyschromia. [9] Traditionally, the management of DH therapy is primarily aimed at occluding the dentinal tubules or making coagulates inside the tubules [10,11]. To achieve this target, the agents must establish a strong lasting adhesion on the smooth surfaces of the teeth [9,12,13].

Desensitizing agents may be used at home (pastes, gels) or in the dental office, but they must fulfill the same requirements: to produce a rapid improvement of DH symptoms [5,6,14,15]. Theoretically, the in-office desensitizing therapy should provide an immediate relief from the symptoms of DH. The in-office desensitizing agents can be classified as the materials which undergo a setting reaction (glass ionomer cement, composites) and which do not undergo a setting reaction (varnishes, oxalates) [17,18].

Conventional bonding agents remove the smear layer, etch the tooth surface and form deep tags inside the dentinal tubules. Resin-dentin layer combined (composed of resinous penetrating tags) is called hybrid layer and insulates effectively the dentinal tubules preventing DH (fig. 1).

The objective of the present study is to determine the adhesion level of various desensitizing agents and the model of hybrid layer on the dental surface. Starting from

* *email: jumanca.daniela@umft.ro*

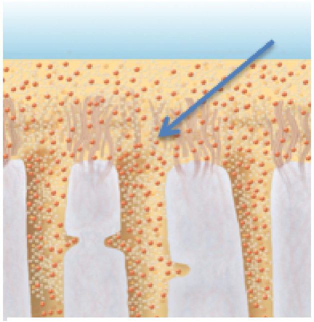


Fig. 1 Graphic representation of the hybrid layer on the dental surface (https://www.dentsply.com/en-us/ restorative /direct-restoration

Fig.2. Samples of the three working groups, initially and after sectioning

the hypothesis that the 3 chosen agents behave identically, this study aims to establish if the composition and presentation of the desensitizing agent determine and influence the hybrid layer and its adhesion.

Experimental part

Material and method

The present study was conducted on 33 human teeth, third molars and premolars, extracted for orthodontic purposes. Consent of each patient was obtained for the proposed study (patient informed consent). Each tooth was sectioned into two halves thus obtaining 66 sections (33 pairs). 33 samples (one half of each tooth) where randomly divided in three groups and treated with a desensitizing agent, after prior demineralization with 37% H_3PO_4 (that simulate a DH tooth with the exposure of the dentinal tubules) (fig. 2). The other 33 samples served as control group after prior demineralization.

Three desensitizing agents available on the market and commonly used in dental offices were used, followed by SEM analysis to observe these desensitizing adhesion types. SEM (scanning electron microscopy) investigation was performed using the FEI Inspect S microscope, in low vacuum conditions to compensate the lack of conductivity of samples and to avoid electrical charge accumulation.

The three substances used in the present study have different chemical compositions but also different physical properties (fluid, paste):

- gluma Desensitizer (Heraeus Kulzer) - Adhesive material;
- fluor Protector (Ivoclar-Vivadent) -Fluoride;
- tooth Mousse (GC) Casein phosphopeptide- amorphous calcium.

Gluma Desensitizer (Heraeus Kulzer, Germania) is a dentinal bonding agent released on the market with the specific purpose to treat DH. This bonding agent contains hydroxyethyl methacrylate (HEMA), benzalkonium chloride, glutaraldehyde and fluoride. Glutaraldehyde causes protein coagulation inside dentinal tubules; it reacts with serum albumin in the dentinal fluid causing its precipitation. HEMA forms deep resin tags and closes dentinal tubules. [10,14]

Fluor Protector (Ivoclar) is a well-known desensitizing agent successfully used in dental medicine offices. It is a varnish containing bis 4-2 difluorhydroxysilil-ethyl-2-metoxi-cyclohexyl, N-N tri methyl-hexane 1,6 dicarbamate, fluorosilane. To increase the product's effectiveness in DH, its acidity was increased. An increased adhesion of fluoride ions is thus obtained, increasing the penetration potential and removing the smear layer [15,16].

GC Tooth Mousse (GC) is a desensitizing agent based upon milk casein. The phosphopeptide casein (PPC) contains phosphoseryl activators which set and stabilize with amorphic calcium phosphate (ACP). This PPC-ACP stabilization prevents calcium and phosphate ions to solve and maintains a supersaturated solution of bioavailable calcium and phosphate. Numerous studies proved that PPC-ACP (Recaldent), can effectively remineralize enamel lesions. In line with its remineralization capacities, it has also been proposed by manufacturers for the prevention and treatment of dentinal hypersensitivity (DH).

In the case of the first two products which are fluid, the samples were dried with the air flow and then the adhesive was applied with a soft disposable brush on the entire demineralized surface where it was left to dry for 10 min. In the case of the third product, the paste was applied with a brush attached to the counter angle for 2 minand then the samples were rinsed with distilled water for 30 s and then dried.

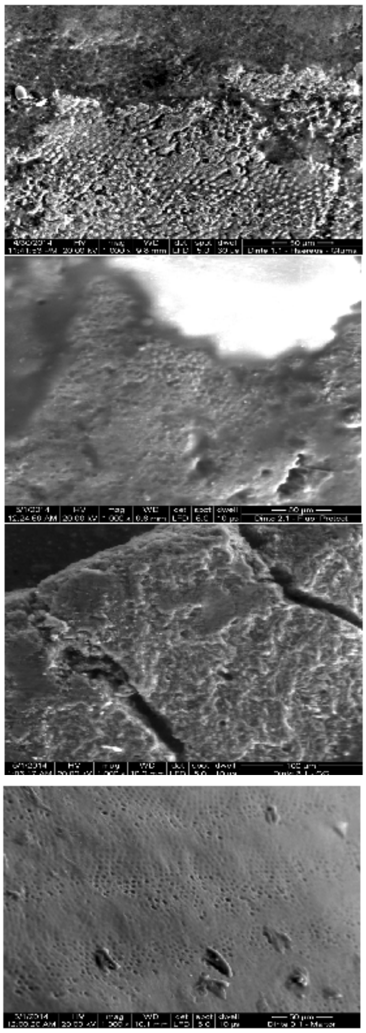
The samples were analyzed with the FEI Inspect S (SEM) microscope, using increasing magnification powers (200, 500, 1000, 2000, 5000x), to assess the even, continuous aspect and the type of adhesion of the hybrid layer for each desensitizing agent.

Analyzing the ultra-structural appearance of the hybrid layer, Tay and Brajdic emphasized three characteristic aspects [7, 8, 17]. The first is the "shag-carpet" aspect of the surface hybrid layer which means loss of the organization of collagen fibrils directed towards the resin adhesive. The second aspect is the hybridization of the canalicular walls and represents the extension of the hybrid layer inside the dentinal tubules. Therefore, the so-called adhesive tags (adhesive retentions) that are formed in the dentinal tubules are circular and are surrounded by a hybrid layer at the canalicular opening. These adhesive tags that penetrate to a distance of 5-10µ from the canalicular opening contribute in the highest degree to the achievement of effective retention and sealing. The third aspect is the so-called "side canalicular hybridization" that has been described as the forming of a thin hybrid layer in the lateral canalicular walls, called *micro-tag,* which surrounds the core of an adhesive extension. [9, 18-20]

Results and discussions

The images in figure 3, illustrate the dental surface covered with desensitizing agent: 3.a Gluma Desensitizer, 3.b Fluor Protector, 3.c Tooth Mouse (GC) and 3.d. compared to 3.d the surface of the control tooth.

Gluma Desensitizer (Hereus Kulzer) belongs to the group of resin-based materials. Resin-based dental adhesive systems can provide a more long lasting dentine desensitizing effect. The adhesive resins can seal the dentinal tubules effectively by forming a hybrid layer [9,10]. The combined dentin-resin layer (consisting of penetrating resinous tags) has been termed as hybrid layer. It effectively seals the dentinal tubules and prevents DH [11,12]. Gluma Desensitizer (Hereus Kultzer), contains hydroxyethyl methacrylate (HEMA), benzalkonium chloride, glutar-aldehyde and fluoride. Glutaraldehyde causes coagulation of the proteins inside the dentinal tubules [13]. Upon SEM



3.a . Gluma Desensitizer, continuous and even hybrid layer, with rare areas of fracture or residual material

3.b. Fluor Protector, the hybrid layer is adhesive but it is not evenly distributed on the work surface and presents numerous anfractuos areas

3.c Tooth Mouse, strong, uneven adhesion, in thick layers with large fracture areas

3.d Control dental surface, gravata acid

Fig. 3. SEM images of dental surfaces covered with desensitizing agents (magnification 1000X), 3.a Gluma Desensitizer, 3.b Fluor Protector, 3.c Tooth Mouse, 3.d control dental surface

analysis of sections on which Gluma Desensitizer (Hereus) was applied, hybrid layer deposits evenly covering the work surface in the shape of an amorphic, continuous and uniform precipitate were observed. The physico-chemical proprieties of the product allow the creation of a continuous and uniform hybrid layer with rare fracture zones or residual material areas (fig 3.a). The adhesion between the material and the hybrid layer is total, uniform, with an increased adhesion degree onto continuous surfaces.

Fluorides decrease dentinal permeability by precipitation of calcium fluoride crystals inside the dentinal tubules [11]. This crystals are partially insoluble in saliva. SEM revealed granular precipitates in the peritubular dentin after application of fluorides. Calcium fluoride crystals precipitate as deposits which adhere to the work surface as an adhesive hybrid layer. Even though the dental surface is covered by the hybrid layer, the latter is not uniformly cast onto the work surface but it presents numerous anfractuous areas, adhesion being achieved in the form of “clusters”, and in certain portions uncovered dentinal tubules may be observed. In images obtained by SEM showing the enamel treated with Fluor Protector (fig.3.b) areas where the desensitizing agent has obliterated interprismatic spaces may be observed, amorphic material areas are revealed, with granular precipitates alternating with areas where the agent has not acted. The adhesion of the hybrid layer formed by Fluor Protector on the enamel surface occured partially but still the adhesive material layer is even and smooth.

SEM images (fig. 3.c) of dental surfaces treated with Tooth Mousse desensitizer based on milk casein show a thick, uneven calcium phosphate layer. Adhesion occurs unevenly, in thick layers with large fracture areas which cause a partial covering of the work surface, with massive deposits on the surface but also vertically, causing the denivelation of the work surface. Phosphopeptidic casein (PPC) contains phosphoserilic activators which set and stabilize with the amorphic calcium phosphate (ACP). This PPC-ACP stabilization prevents the solubilization of calcium and phosphate ions and maintains a supersaturated solution of bioavailable calcium and phosphate. Numerous studies demonstrated that PPC-ACP (Recaldent) can effectively remineralize enamel lesions. Due to its remineralizing capacity, it has also been proposed by manufacturers for the prevention and treatment of dentinal hypersensitivity.

Figure 3.d shows the control sample, used to compare the dental surface with exposed dental tubules, without hybrid layer with dental surfaces in the work group.

By comparative analysis of the SEM images of the 33 treated samples, we observed differences regarding the aspect of the layer and its adhesion on the dental surface. (table 1.)

The application of the desensitizer on dental surfaces with the purpose to treat DH is directly achieved on dental surfaces exposed in the oral cavity both on the enamel and on dentine and/or cementum; thus, the adhesion of the material depends on the physico-chemical proprieties of the desensitizing agents and of the hybrid layer. The application of the desensitizing substance must ensure a complex adhesion process to all surfaces so that the effect to be immediate and durable.

The adhesion of the desensitizing agent is achieved by the formation of the hybrid layer on the dental surface and by its physical-chemical capacity to form micro-tags. The

Tabel 1
REZULTS BY COMPARATIVE ANALYSIS OF THE SEM IMAGES OF THE 33 TREATED SAMPLES

37 teeth					
66 samples					
33 study samples			33 martor samples		
1. Gluma Bond	2. Fluor Protector	3. Tooth Mouse	M1	M2	M3
+++	++++	++	0	0	0
++++	++	+	0	0	0
++++	+++	++	0	0	0
+++	++	+++	0	0	0
++++	+	++	0	0	0
++	++++	+	0	0	0
++++	++	+	0	0	0
+++	+++	++	0	0	0
++++	++	+++	0	0	0
+++	+	++	0	0	0
++	++	+	0	0	0

+ + + + very good addesion, strat hibrid uniform ; + + + good addesion, rare denivelari in starul hibrid; + + satisfying addesion, denivelari in stratul hibrid; + low addesion, strat hibrid neuiniform, zone de fractura ;0 control sample, demineralised surface opening of dentinal tubules and protein precipitation inside dentinal tubules must occur in a moist environment, without removal of the smear layer, on the entire dental surface, evenly, in a thin pellicle which does not represent a plaque retentive factor. [10,11,21] After application of the three desensitizing substances we observed that Fluor Protector ensures an uneven hybrid layer on the work surface, with numerous anfractuous areas, the adhesion occurring in the form of *clusters*, with uncovered dental canaliculi in certain areas. The physical-chemical proprieties of Gluma Desensitizer allow the creation of a countinuous and even hybrid layer, without fracture areas or residual material. The adhesion of the material allows the achievement of an even hybrid layer with a high degree of adhesion on the dental surfaces. Tooth Mousse, based on milk casein, determines the formation of a thick, uneven calcium phosphate layer. Adhesion occurrs unevenly, in thick layers with large fracture areas, which leads to partial covering of the work surface, with massive deposits on the surface but also vertically, leading to a denivelated work surface [22].

Fluor Protector, Tooth Mousse and Gluma Desensitizer ensure adhesion to dental surfaces, with formation of the hybrid layer and micro-tags, but the level of adhesion varies depending on the physical-chemical proprieties of the product [22, 23].

The hybridization of the dentine wound is a process that creates a hybrid at molecular level, i.e. an area with physico-chemical properties which differ from the original local structure through partial demineralization and impregnation of the collagen fibers exposed with polymer resin adhesive [8,25,26]. The dentin detritus called smear layer covers the surface of any dentin wounds and it is the result of the process of physicochemical degradation of the proteins from the heterogeneous structure of the hard dental tissues [12,14,24].

Conclusions

The action of desensitizing agents to close dental tubules and interprismatic spaces on affected dental surfaces is effective, based on their adhesion proprieties, but the resulting layer is not even in all products. Comparing the three products used in the present study, based upon electron microscopy images (SEM), it was concluded that Gluma Desensitizer (Hereus Kulzer) leads to the most homogeneous hybrid layer on the dental surface, followed by Fluor Protector (Ivoclar) and Tooth Mouse (GC). The adhesion process is influenced by the presentation of the desensitizer: fluid or paste and by the type of physicochemical reaction that leads to the formation of the hybrid layer.

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