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

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The objective of the present study was to determine the adhesion level of various desensitizing agents and to establish a model for the hybrid layer on dental surfaces. Also, this study aims to test the composition and presentation of the desensitizing agent in order to 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 properties. 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 hypersensitivity, electron microscopy, desensitizing agents, hybrid layer, adhesion

Modern lifestyle has led to various dental diseases, other than 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].

Bränström, 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 dental tubules by specific adhesion of certain substances to smooth dental surfaces.

Grossman formulated the requirements for an agent to achieve an ideal dental 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

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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% H₃PO₄ (that simulate a DH tooth with the exposure of the dentinal fluid causing 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 amorphous 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 min and 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 Mousse (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 Kulzer), 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]
After application of fluorides, calcium fluoride crystals precipitate as deposits which adhere to the工作表面 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 dental 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, amorphous 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 occurred 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 amorphous 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

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Tabel 1
RESULTS BY COMPARATIVE ANALYSIS OF THE SEM IMAGES OF THE 33 TREATED SAMPLES
of Dipl Ing. Dr. Cosmin Locovei, IMF Departament, Polytechnical University Timisoara.

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References

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