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IN VITRO STUDY OF DENTIN HYBRID LAYER OF A NEW RESIN COMPOSITE MATERIAL: COMPARISON BETWEEN THE USE OF DIAMOND AND ER, CR: YSGG LASER CAVITY PREPARATION

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The aim of this study is the analysis by Scanning Electrono Microscopy (SEM) of hybrid layer thickness cavities prepared with diamond burs and with Er, Cr: YSGG laser, filled with two resin composite materials with low viscosity Smart Dentin Replacement (SDR™) by DENTSPLYDeTrey-Konstanz- Germany and Tetric®flow (T®f) by Ivoclar-Vivadent-Liechtenstein. The study was realized *in vitro* on samples of 20 human premolar and molar teeth extracted for orthodontic or periodontal reasons, divided at randomly into four groups (Gr.1 to 4) of 5 teeth. The teeth were prepared and restored according to the manufacturers instructions. The specimens were prepared for SEM observation and analyzed using ANOVA tests ($p \leq 0.05$). The resulting thicknesses has highlighted differences between groups ($p \leq 0.05$): Group1:17.27(±8.12)µm>Group3:14.08(±7.58)µm>Group2:8.77(±4.44)µm>Group4:6.51 (±1)1 µm. In conclusion, the preparation technique and the material used determine the thickness of the hybrid layer in favor of diamond cavity preparations filled with SDR™.

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1. Introduction

Adhesion of dental materials to dental substructures is an essential condition for the durability of restorations. This is dependent on a number of related factors: dentin substrate characteristics, the type of approach to dentin substrate and the type of material used [1,2].
The quality of the resin-tooth biostructure interface determines the integrity and durability of the adhesive restorations [3-6].

Closing marginal at the gingival threshold level is difficult. In these circumstances the choice of the material to promote adhesion to dentin is current. SDR™ is a new flowable composite resin material recommended as dentin substitute. SDR™ is a single component, fluoride containing, visible light cured, radiopaque resin composite restorative material. It is designed to be used as a base in class I and II restorations. In this context our study has suggested analysis of penetration of this material in dentin substructure compared with another material applied in class II cavities. On the other hand, dental substrate preparation tends to be minimally invasive. Among minimally invasive techniques include and kinetic preparation technique with laser.

Laser (L) cavities preparation allow to ablate the cariouse tissues and to modify the dentinal structure, thus increasing the free surface energy and surface roughness. Although laser irradiation offers many micro-irregularities, it was observed that the dentinal layer does not allow

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an optimal penetration of the material [7]. Furthermore, the erbium laser may affect the degree of the mechanical anchorage, as the bonding resin can infiltrate into the irregularities of laser-treated dentine. These micro-irregularities have been reported to occur due to the termomechanical ablation process with laser which causes micro-explosions in areas of high water concentrations and on the hydrated part of hydroxyapatite.

Other studies concluded that the erbium core laser has a negative influence on the formation of a hybrid layer and cavity preparation methods influence the formation of the hybrid layer [8,9].

The purpose of this work is the study of hybrid layer thickness in Class II cavities prepared mechanically and kinetically- laser and filled with two resin composite materials with low viscosity by using SEM.

2. Experimental

Twenty caries-free molars and premolars were extracted from orthodontically and periodontologically reasons. The teeth were obtained from patients who required an extraction as a routine part of their treatment. The research was conducted with the agreement of the Ethics Commission of the Iasi University of Medicine and Pharmacy (UMF Gr.T. Popa).

The samples were randomly divided into four equal groups. The teeth were mechanically brushed with a non-fluorurate abrasive paste, rinsed with dionised water and stored in 0.5% chloramine solution at 4^0 C. Class II standard cavities were performed at each tooth: 4 mm high at 1 mm distance from enamel-cement jonction, 4 mm width, 2 mm depth mesial and distal, ten cavities for each technique (N=10). Cavities were performed M (mechanically) and L (kinetically) irradiated with Er,Cr,:YSGG laser.

Mechanical preparation was done at slow and high speed under water spray with round and cylindrical diamond burs no:1. Kinetical preparation with Er,Cr:YSGG laser (Biolase Waterlase-MD) was done with MG6-MZ6 tips. The working parameters for Er,Cr:YSGG laser were, according to manufacturer's instructions, as follows: for enamel 30% water and 60% air at 5.5 W/ 20 Hz and for dentin 30% water and 60% air at 3W/15 Hz.

The materials used for filled the teeth were adhesive system with SE™ (SchotchbondEtch-3MESPE-Seefeld, Germany) with ASBP (AdperSingleBondPlus-3MESPE Seefeld, Germany) wich contain silica nanofiller Bis-GMA, HEMA, dimethacrylates, ethanol, water, a novel photo initiator system and a methacrylate functional copolymer of poly-acrylic and polyitaconic acids, resin composite Smart Dentin Replacement (SDR) provided by DENTSPLY DeTrey Konstanz-Germany, Tetric®flow (T®f) provided by Ivoclar-Vivadent-Liechtenstein and halogen lamp (QTH), power density 570mW/cm^2 (3MESPE). The teeth were prepared and restored according to the manufacturers instruction as follows: Group 1 - M and filled with SDR; Group 2 - L and filled with SDR; Group 3- M and filled with T®f; Group 4- L and filled with T®f. Cavities were filled in incremental layers 4 mm-SDR™ and 2 mm-T®f.

The samples were cut longitudinaly (mesio-distal) with diamond disc under water cooling and then washed with ethanol. Then the slices were processed with 400, 600, 1200 and 2400 grit SiC paper, polished with gums and abrasive paste 6, 3, 1 and 0,25 μm under continuous irrigation. The samples were etched with H_3PO_4 35% for 4 seconds and washed for two minutes with distilled water as presented in Ref. 6 [10]. The teeth were then stored in saline solution 48 hours. SEM observation was done with JEOLJSM 6390[a] Japan.

Hybrid layer thickness was characterized by SEM using a JEOLJSM 6390 microscope. The measure was performed at the lateral and gingival walls of the restauration in at least three different points, making the average for each sample. The Analysis of Variance (ANOVA) with Bonferroni test was used to establish a 95% confidence interval.

3. Results

The obtained statistical mean of thicknesses of hybrid layer, for the four test groups, are as follows: Group1=17.27(±8.12)μm, Group 2=8.77(±4.44)μm, Group 3=14.08(±7.58)μm, Group

4=6.51(±1.15)µm. The highest thickness and therefore the best results were obtained for Group 1, the conventional samples (M) filled with SDR™. The equality of variances test (Levene test) was positive at the p-value of *p* =.000 with semnificative differences between the averages. The ANOVA tests showed statistically significant differences between the groups showing that the preparation technique and the restoration material influence the dimensional level of hybrid layer. Bonferroni test was used to evidence the differences betweeen the groups: SDR™ *p* =.019, also for T®f, *p* =.047 (Table 1)

Table 1 Multiple Comparisons between hybrid layer in study groups

Dependent Variable: HL

Bonferroni

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GR.1(M)SDR™-D	GR.2(K)SDR™-D	8.50(*)	2.69	.019	.99	16.01
	GR.3(M)T®f -D	3.20	2.69	1.00	-4.31	10.71
	GR.4(K)T®f -D	10.77(*)	2.69	.002	3.26	18.28
GR.2(K)SDR™-D	GR.1(M)SDR™-D	-8.50(*)	2.69	.019	-16.01	-.991
	GR.3(M)T®f -D	-5.30	2.69	.34	-12.81	2.21
	GR.4(K)T®f -D	2.27	2.69	1.00	-5.24	9.78
GR.3(M)T®f -D	GR.1(M)SDR™-D	-3.20	2.69	1.00	-10.70	4.31
	GR.2(K)SDR™-D	5.30	2.69	.34	-2.21	12.81
	GR.4(K)T®f -D	7.60(*)	2.69	.047	.06	15.08
GR.4(K)T®f -D	GR.1(M)SDR™-D	-10.77(*)	2.69	.002	-18.284	-3.26
	GR.2(K)SDR™-D	-2.27	2.69	1.00	-9.78	5.24
	GR.3(M)T®f -D	-7.60(*)	2.69	.047	-15.08	-.06

* The mean difference is significant at the .05 level. HL=Hybrid Layer, GR=group, M=Mechanicaly, K=Kinetic with LASER, D=dentine.

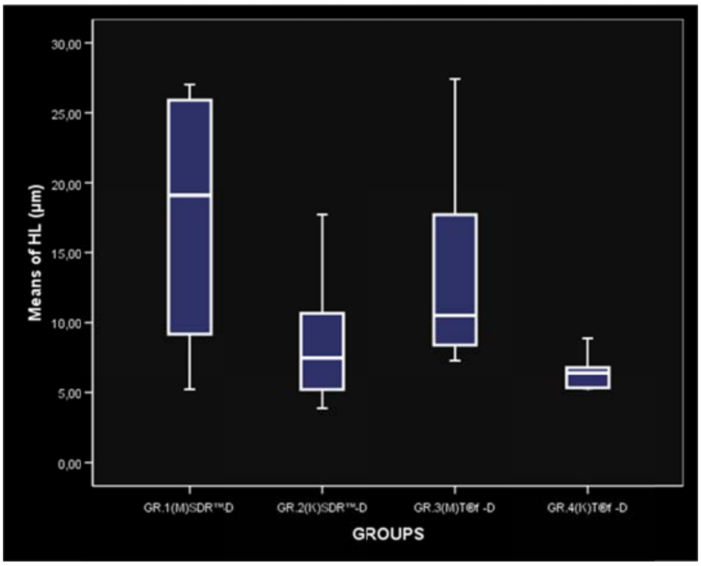

Fig. 1. Level of distribution symmetry in the groups (hybrid layer measurements are in µm)

There were no significant differences in hybrid layer thickness for the same preparation technique, but filled with different materials (*p* =1.000).

Differences between the groups are listed in Table 1 and shown in Fig.1.

Although the micrograph analysis did not show significant differences between the groups. The hybrid layer for SDR™ is continous, while the T®f interface evidenced an irregular hybrid layer with multiple disarrangements, especially at gingival margins of the restaurations for both samples M (Fig.2 and Fig.4) and L (Fig.3 and Fig.5).

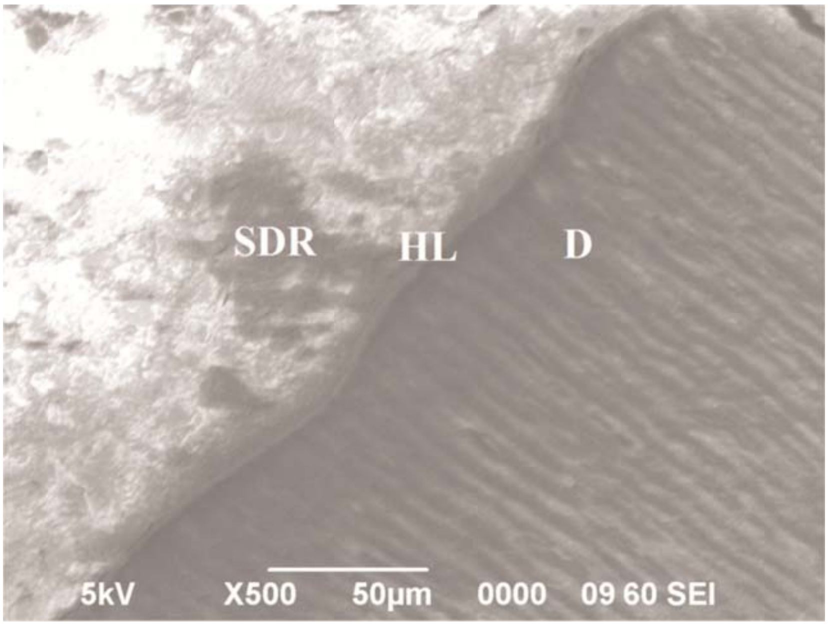

Fig. 2. SEM micrograph of dentine – material interface in conventional cavities M. A constant hybrid layer is present, determined by the penetration of the SDR™ material through the dentinal tubules. The disposal of the dentinal canalicules is perpendicular to the interface layer.

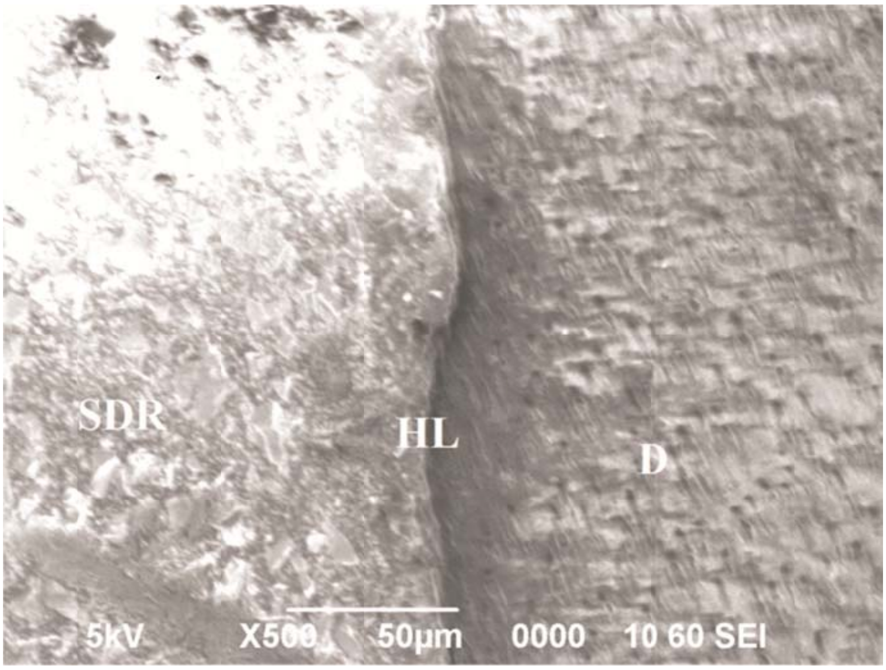

Fig. 3. SEM micrograph of dentine – material interface in laser ablation cavities K, hybrid layer dimension is diminished, but regular throughout the interface, due to the penetration of the SDR™ material. The oblique disposal of the dentinal tubules on the lateral walls reduces the dimension of the hybrid layer.

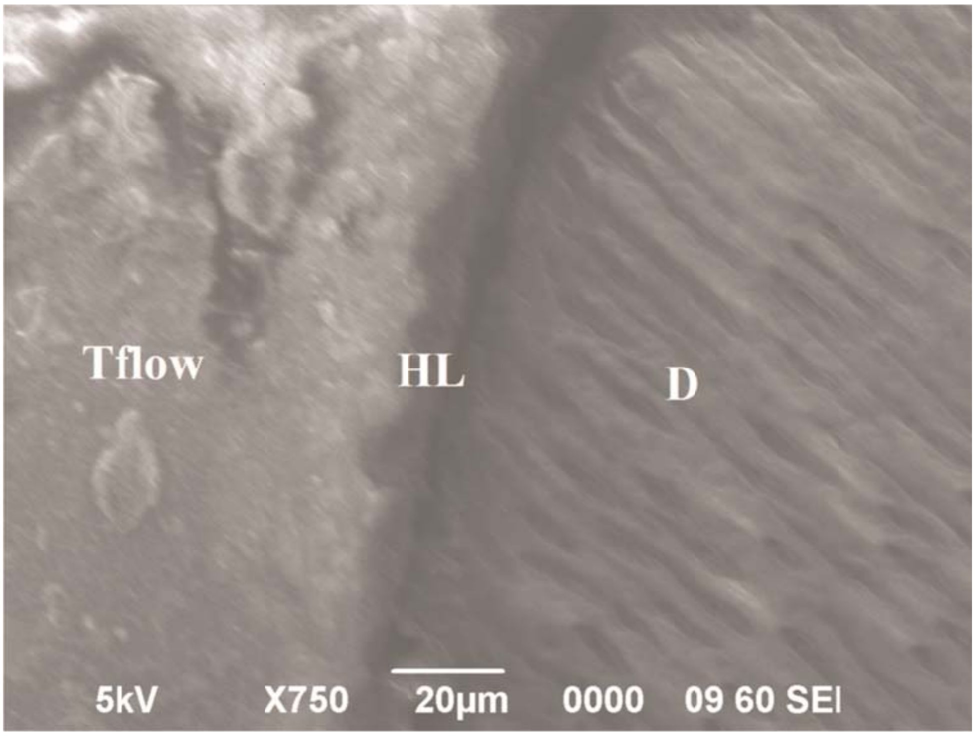

Fig. 4. SEM micrograph image of dentine – material interface in conventional cavities M. The hybrid layer dimension is irregular, due to the penetration of the T®f material.

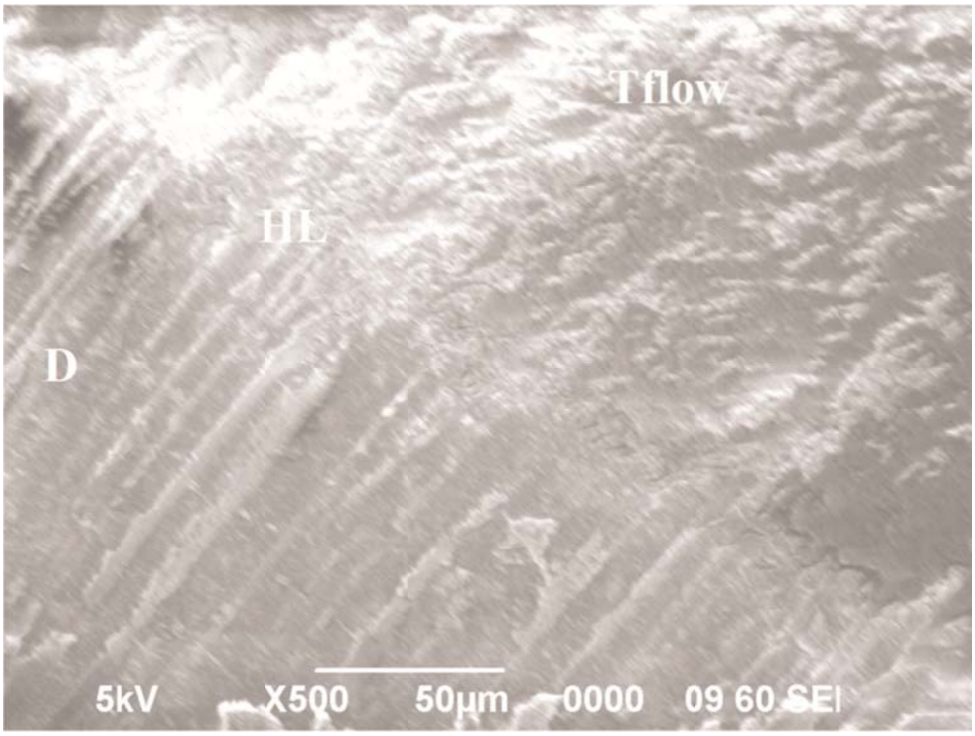

Fig. 5. SEM micrograph image of dentine – material interface in LASER ablation cavities K, on the lateral dentine walls. The hybrid layer dimension is reduced but regular, due to the penetration of the T®f material.

SEM micrograph analysis of hybrid layer showed that the best results were obtained for the M samples, filled with SDR™. We also obtained good results at the internal angle between the vertical and the horizontal wall from the gingival margins of the restauration. The lowest values and highest dissarengements were found at the gingival margins.

4. Discussions

The present study finds significant statistical differences between the means of the hybrid layer thickness, in favor of those prepared conventionally (mechanically). This demonstrates why the mechanical preparations used with an adhesive system etch and rinses SE™; ASBP cause a clean substructure with no smear layer (Fig.2 and Fig.4).

Similar results were obtained by Giachitti [11] using T®f, for a 10 micrometers hybrid layer.

The hybrid layer thickness shows significant statistical variations from group to group, but also within each given group, as reported by other groups [12-14].

With a hybrid layer partially infiltrated, the original samples can have a 5µm hybrid layer, which, after the exposure to acid and bases, decreases to only 3 µm. This shows that, the first 2 mm of the hybrid layer have been incompletely infiltrated by monomers or that the hybrid layer surface was not well polymerized because of the acid/base treatment.

The hybrid layer resistance against chemical assault depends on the materials used to infiltrate the pretreated dentin and on the type of the polymerization [15].

The incomplete infiltration present in similar samples may have numerous causes, for instance: the desiccation of the collagen network who did not expand homogeneously after the primer application, thus closing spaces between the dentinal tubules; and reduced time for the demineralization of the collagen network [16]. In some cases, the collapsed demineralization area forms a hybrid layer which superficially obliterates the opened dentinal tubules, allowing the resin to infiltrate into the lateral tubules.

Etching with orthoposforic acid 35% can produces transitory modifications of dentine, from partial demineralization to total loss of the hydroxiapatite crystals, as a function of etching time. After conditioning the dentin with orthoposforic acid 37% we obtain two phases of demineralization: phase one, the superficial dentin layer which is the decalcification of the dentin with the exposal of the collagen fibres, and phase two, the gradual demineralization of the dentin continuing the undamaged dentin. The adhesives system used in this study, Adper Single Bond Plus contain two acrylic monomers Hydroxyethylmethacrylate (HEMA) and Bisphenol A-Glycidyl Methacrylate (Bis-GMA). The organic component (which includes HEMA) of the Adper Single Bond Plus causes the humectation of the dentinal substructure during the phase two; meanwhile Bis-GMA can not penetrate into the profound areas because has a high viscosity.

In such situations, the dentinal substructure still remains protected from the secondary carious lesions because of the hybridization process. The hybrid layer microstructure depends on the dentin water quantity, since water around collagen fibrils causes insufficient monomer infiltration. This causes a contraction of the demineralized dentin after an exigent desiccation, which affects the hybrid layer structure. After contraction, the dentinal tubules are modified in dimensions and interfere with the formation of the regular hybrid layer. When we analyze the morphology of the hybrid layer, we have to take into account the dentinal structure. It is important to note that perpendicular tubules could determine a thicker hybrid layer and that in sclerotic dentin the hybrid layer may be absent.

The viscosity of the material can play an important role in the formation of the hybrid layer. The materials we used have similar degrees of viscosity: SDR™ with 68% by weight, 45% by volume and T®f with 67% filler by weight, 43% by volume. A lower viscosity favorizes hybrid layer humectation but increases the polymerization contraction. Although we used an adhesive system with a high bonding strenght of 25-30 MPa, through polymerization contraction, some marginal leakages appeared due to the sealing material ASBP. This was revealed in other studies respectively 55% (39-70) at the dentin under pulpal pressure, 77% (68-83) at the desicated dentin and 41% (27-65) for the wet dentin compared to 48%, 42% and 38% with Seal Protect (Dentsply DeTrey) [17].

Meanwhile, we observed that the resin-dentin interfaces average was variable as it was also revealed in other studies: 9.83 μm or 5 μm [12,18]. This difference can be inferred to the fact that the resin penetrates only very slightly on the dentinal tubules.

Another important aspect we need to take into account in our SEM analysis, is the shrinkage of the hybrid layer due to dessication during the SEM examination process. This phenomena provided evidence of the presence of the demineralized dentin zone within the hybrid layer [19]. The two investigated composite materials gave similar results regarding the interface quality and the marginal adaptation in dentin. The conventional resin cement associated with the etch& rinse adhesive systems gave the lowest microleakage values and formed the most homogeneous interface between the composite inlay and dental biostructure [20].

The success of a good restauration is gained by marking the properties of the hybrid layer in order to preserve the dental pulp. Besides the differences in the materials used, the preparation techniques could influence the bonding of resins.

More studies are needed to test the power of adhesion of the materials used and tracking clinical behavior of these methods.

5. Conclusions

Within the limits of the present study, it can be concluded that the preparation technique and the material used determine the thickness of the hybrid layer. All samples prepared by laser features a lower hybrid layer than at samples prepared mechanically. There are not major differences between the samples prepared with the same technique, mechanical or kinetic-laser and restored with different materials. The best result was obtained for the mechanical samples filled with SDR™.

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