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MICROSCOPIC EVALUATION OF THE INTERFACE BETWEEN COMPOSITE BIOMATERIALS AND DENTIN BIOSTRUCTURE

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This study is dedicated to the investigation of the interfaces between the dentin biostructure and composite inlays manufactured using the trade-mark Gradia and the experimental Barodent biomaterials. The restorations were luted with a self-adhesive resin cement and with a conventional resin cement associated either with a two-step etch & rinse adhesive or with a three-step etch & rinse adhesive. The scanning electron microscopy examination and the optical microscopy coupled with the microleakage investigations, followed by a statistical analysis of data, allowed the qualitative and quantitative evaluation of the interface between composite biomaterials and tooth biostructure, as influenced by the restoring material and the resin cement. 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.

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1. Introduction

Resin-based composites are the most frequently used biomaterials in restorative dentistry especially due to the increased aesthetic demands [1-5]. The progress made during the last decades regarding the restorative and bonding techniques proved that direct and indirect composite restorations can be successfully used in dentistry [2- 4]. The quality of the resin-tooth biostructure interface determines the integrity and durability of the adhesive restorations[6-9].

The interface between the restorative biomaterials and the dental substrate represents an interdiffusion zone that offers bonding sites for copolymerisation with composites, meanwhile acting as a protective layer for the tooth, blocking microorganisms and toxins [10, 11]. For an adequate marginal adaptation of restorations, a strong and durable bond to the tooth biostructure is necessary [12-14].

For direct or indirect composite restorations, bonding to different tooth substrates is variable. Enamel is a reliable substrate for bonding while dentin remains less reliable or predictable due to its structural characteristics i.e. the high organic content, the tubular structure and the presence of an outward fluid movement [12, 15, 16].

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The bonding interface consists of multiple structures such as bonding resin, hybrid layer and dentin substrate [17]. Their characteristics influence the future durability of the bonding, and consequently of the restoration [18]. The failure of the bonding interface allows the microleakage of bacterial enzymes, oral fluids and bacteria between the tooth and restoration, leading to recurrent decay, hypersensitivity, pulpal inflammation and finally to restoration loss [19]. Achieving a perfect marginal quality with composite inlays, when gingival margins are located in dentin, continues to be critical even when new adhesive techniques and systems are used [19].

Scanning electron microscopy (SEM) and dye penetration tests represent valid tools for the evaluation of the marginal integrity in *in vitro* studies [20]. SEM is a widely-used method in the morphological examination of bonded interfaces produced by adhesive systems to various tooth substrates. Additional investigations such as dye penetration or microleakage studies are used to obtain a quantitative evaluation of the extent of the marginal gaps [15,20].

The present study continues our researches referring to the use of different biomaterials for inlay restorations [5-8]. The aim of this paper is to investigate the adhesive interfaces created by different composite biomaterials inlays with the tooth biostructure. The quality of the marginal adaptation achieved with the materials on trial was assessed *in vitro* through microleakage testing and scanning electron microscopy observation of the tooth-cement-inlay restoration interface.

2. Experimental

The *specimen preparation* was achieved using several biomaterials. The inlay restorations were applied on extracted teeth and they were tested *in vitro*. Indirect composite inlays were manufactured using a trade mark material –Gradia, produced by GC Corporation, Tokyo, Japan, and an experimental material Barodent, produced by Raluca Ripan Institute for Research in Chemistry ICCRR, Babes Bolyai University Cluj-Napoca, Romania. The indirect restorations were luted with different resin cements. The restorative materials, resin cements and adhesive systems used in this study and their main characteristics are presented in table 1.

The investigation was carried out using sixty sound permanent third molars. Two standardized class II cavities were prepared on each tooth according to the standard protocol for indirect restorations. The cervical limits were placed in dentin at 1 mm below the cemento-enamel junction (CEJ). Teeth were randomly divided into 6 groups (n=20) and restored following manufacturer's instructions. Groups 1, 3, 4 were restored with Gradia composite inlays which were luted using different resin cements i.e. G-Cem (GC Corp.) for G1, Excite DSC+ Variolink II (Ivoclar-Vivadent, Schaan, Liechtenstein) for G3 and Optibond FL (Kerr Corporation, Orange, USA)+Variolink II for G4. Groups 2, 5, 6 were restored with Barodent composite inlays which were luted with the same resin cements, following the above mentioned sequence i.e. G-Cem for G2, Excite DSC+Variolink II for G5 and Optibond FL+Variolink II for G6.

The restored teeth were kept in distilled water at 37°C for 24 hours and then they were thermocycled for 2000 cycles (MJ Minicycler), between 5°C and 55°C with a dwell time of 30 seconds and a transfer rate of 10 seconds between each bath. The teeth were immersed in a 2% methylene blue solution for 24 hours then rinsed for about 10 minutes in running water. They were sectioned in the mesio-distal direction using a low speed diamond saw (Isomet, Buehler Ltd.) resulting one section of 1mm width in the middle of the restoration.

The specimens were analysed by *Scanning Electron Microscopy* (JEOL-JSM 550LV Microscope) in order to evaluate the quality and continuity of the restoration-tooth biostructure interface. The illustrative SEM images were selected at the most appropriate magnification for every investigated system. Dye penetration at gingival margin was examined by *Optical Microscopy* (Olympus KC301, Olympus America Inc.) at 40x magnification. The microleakage values (μm) were recorded using a QuickPhoto Micro 2.2 software (Olympus Inc). *Statistical Analysis* was performed using Kruskall-Wallis and Mann-Whitney tests at a $p<0.05$ level of significance, with SPSS 13.0 and Statistica 7.0 software [21].

3. Results

This study is dedicated to the investigation of the interface between the dentin biostructure and composite inlays that were manufactured using the trade-mark Gradia and the experimental Barodent biomaterials (Table 1). The restorations were luted as follows: a) with the self-adhesive resin cement G-Cem, in specimen groups G1 and G2; b) with the conventional resin cement Variolink II associated with the two-step etch & rinse adhesive Excite DSC in specimen groups G3 and G5; c) with the conventional resin cement Variolink II associated with the three-step etch & rinse adhesive Optibond FL, in specimen groups G4 and G 6.

Table 1.Short characterisation of the biomaterials on trial.

Product/ Manufacturer	Type	Characteristics
Gradia/GC Corp	Composite material	*Hybrid filler* (75 wt%): silica micro-filler mixed with UDMA resin, heat-polymerized and grounded in small particles (10-50 μm); silanised ceramic fine particles (<2 μm) *Organic matrix:* UDMA (urethane dimethacrylate) based resin
Barodent/ ICCRR	Composite material	*Hybrid filler* (65 wt.%): barium glass (50%); colloidal silica (20%);quartz (30%) *Organic matrix:* Bis-GMA Resin (Bis-phenol A diglycidyl methacrylate), TEGDMA (triethylene glycol dimethacrylate) and UDMA based resin
G-Cem /GC Corp	Self-adhesive dual-curing resin cement	*Powder:* Fluoro-alumino-silicate glass, initiators *Liquid:* Functional monomers: 4-MET (4-methacryloyloxyethyl trimellitic acid) and phosphoric acid ester monomers
Variolink II/ Ivoclar-Vivadent	Conventional dual-curing resin cement	*Paste:* mixture of dimetacrylates (Bis-GMA, UDMA, TEGDMA); inorganic filler; ytterbium trifluoride
Excite DSC/ Ivoclar-Vivadent	Two-step etch & rinse adhesive system	*Etchant:* 37% phosphoric acid *Adhesive:* HEMA (2-hydroxyethyl methacrylate), phosphonic acid acrylate, mixture of dimethacrylates, SiO2, alcohol
Optibond FL/ Kerr	Three-step etch & rinse adhesive system	*Etchant:* 37.5% phosphoric acid *FL Primer*: HEMA, GPDM (glycerol-phosphate dimethacrylate), MMEP (mono- 2-methacryloyloxyethyl phthalate), water, ethanol *Adhesive*: Bis-GMA, HEMA, GDMA (glycerol dimethacrylate), filler (fumed SiO2, barium aluminoborosilicate, Na2SiF6)

By investigating the interaction between the dentin biostructure and different biomaterials, useful data were collected regarding the interface characteristics and the marginal adaptation of restorations.

The *in vitro* analysis using SEM and optical microscopy is useful to assess the quality of the investigated interface and to predict the clinical performances of the investigated biomaterials.

3.1 Qualitative examination

The scanning electron microscopy was used for the microstructural characterisation of the adhesive interface that is the interaction between the composite inlays and the dental substrate in correlation with the adhesive system/resin cement used for the luting procedure.

SEM images for specimens in Group 1 (Fig.1a) illustrate the formation of a rather continuous interface between the resin cement and the dentin. The micrographs for specimens in Group 2 (Fig.1b) showed similar structural characteristic as for the specimens in Group 1, as they used the same resin cement. For both specimen groups, the presence of some gaps along the adhesive interface could be observed at higher magnifications. For specimens in Groups 3 (Fig.1c) and 5 (Fig.1e) which used a conventional resin cement with a two-step etch& rinse adhesive, the micrographs showed a continuous resin-tooth interface for most of the specimens, with the formation of a uniform hybrid layer. Similarly, for the specimens in Groups 4 (Fig.1d) and 6 (Fig.1f) that used a conventional resin cement with a three-step etch& rinse adhesive, the SEM images showed a continuous and uniform adhesive interface between the resin cement and dentin biostructure, underlining the good interaction between the two substrates.

For all specimens groups, a homogeneous and continuous interface between the resin cement and the composite inlay was observed, thus underlining the good compatibility between these biomaterials.

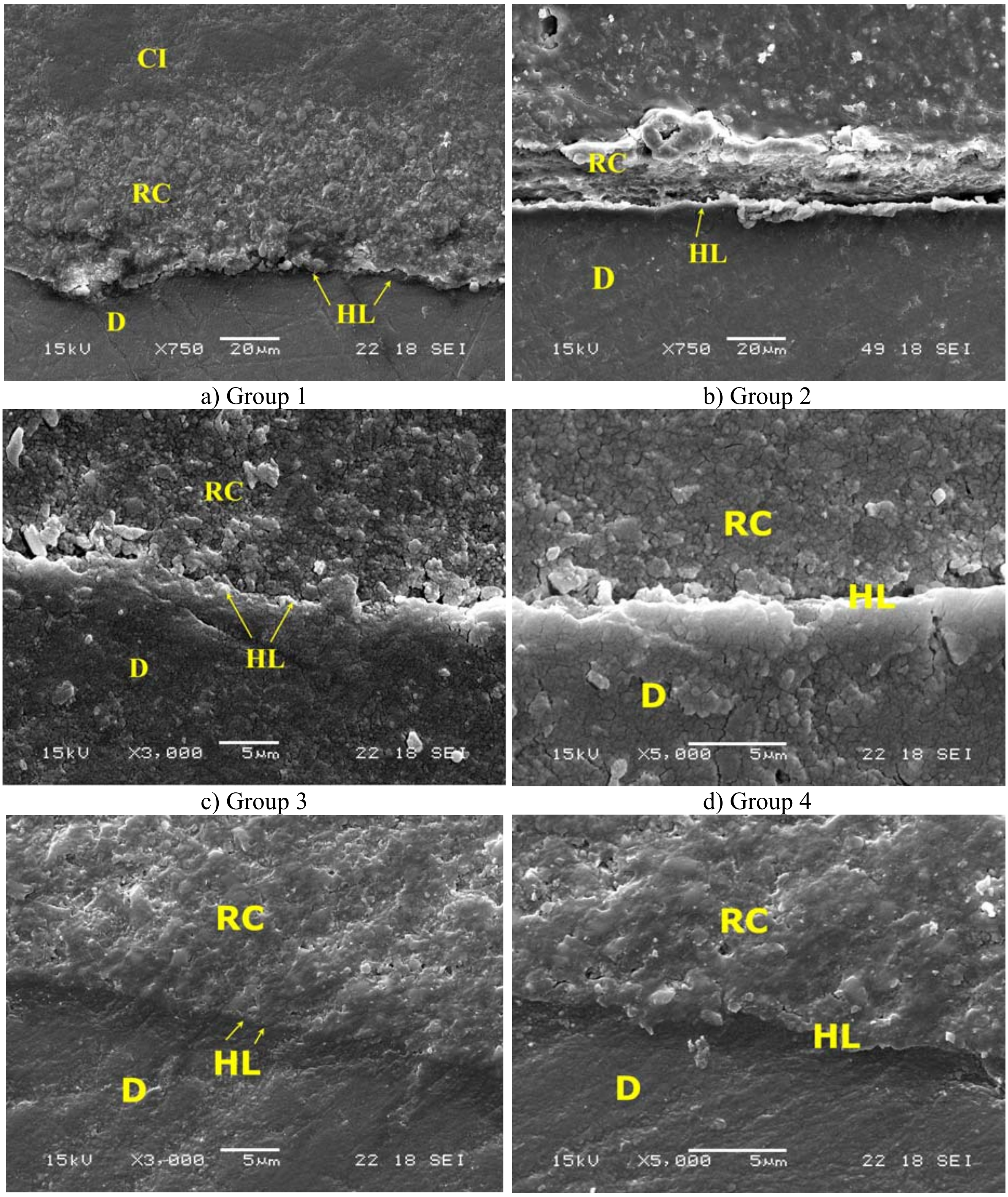
Scanning electron microscopy allows a qualitative evaluation of the adhesive interfaces created between the dentin biostructure and the inlays prepared from two different materials luted with different resin cements and adhesive systems.

3.2 Quantitative analysis

The investigations on the marginal adaptation of restorations are usually performed by microleakage tests, by appreciating the degree of penetration of different staining dye solutions in which the specimens are immersed. In this study, the marginal leakage along the adhesive interface of composite inlays was evaluated by optical microscopy, after the specimen immersion in 2% methylene blue solution.

A quantitative analysis was carried out based on the measurement of the length of dye penetration. The optical microscopy images illustrate the marginal microleakage in dentin and enamel, which is marked with a green line, in relation with the total length of the restoration-tooth interface that is marked with an orange line. In some cases, the width of the resin cement is also marked with pink line (Fig.2). The length of the tooth-restoration interface and/or length of microleakage were calculated, by addition because the entire specimen was not visible on the same image. The microleakage proportion was evaluated by the ratio between its length to the length of the tooth-restoration interface.

The specimens groups restored using the same composite material were statistically analysed using the Kruskall-Wallis test which showed significant differences between them ($p<0.05$), the level of significance for the proportion of microleakage in dentin being $p= 0.00001$ for the specimens restored with Gradia composite inlays (G1,G3,G4), and respectively $p=0.0002$ for the groups restored with Barodent inlays (G2, G5, G6).

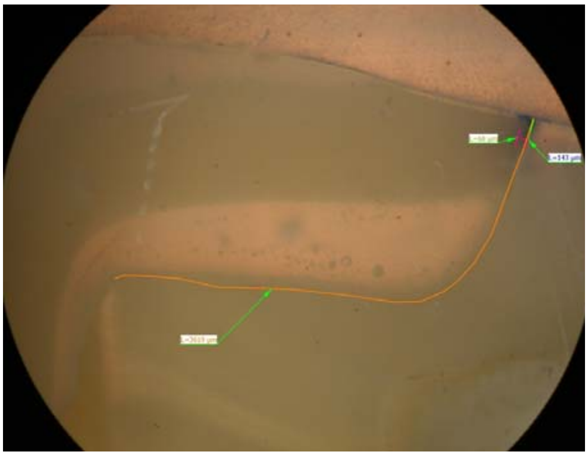


a) Group 1 b) Group 2

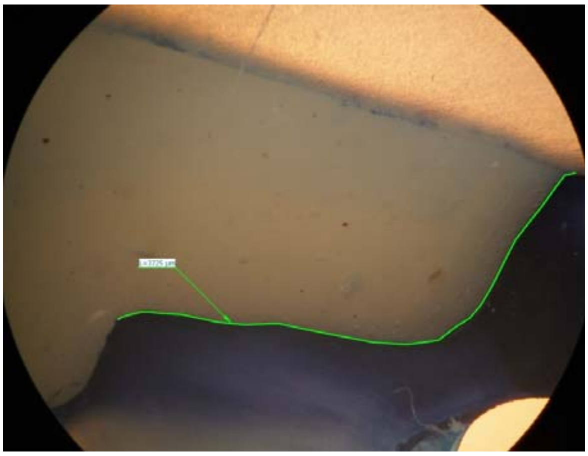
c) Group 3 d) Group 4

e) Group 5 f) Group 6

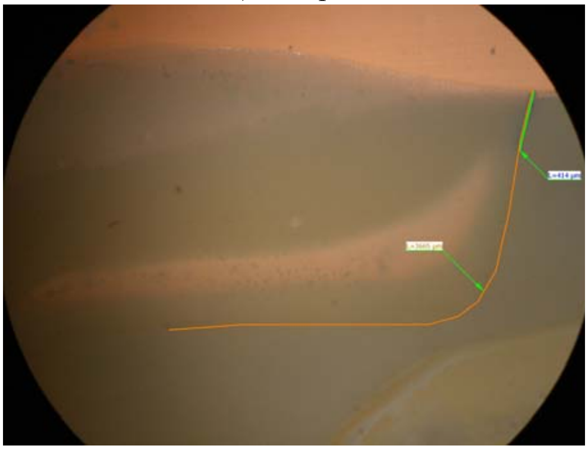
Fig.1 SEM micrographs showing the adhesive interface of Gradia (a,c,d) and Barodent (b,e,f) inlays, where CI-composite inlays, RC-resin cement, HL-hybrid layer, D-dentin



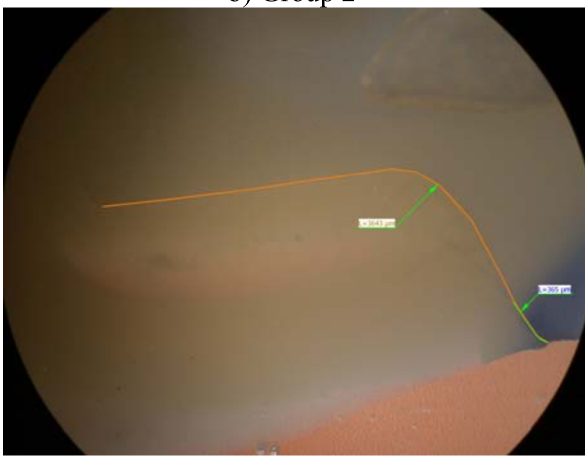
a) Group 1



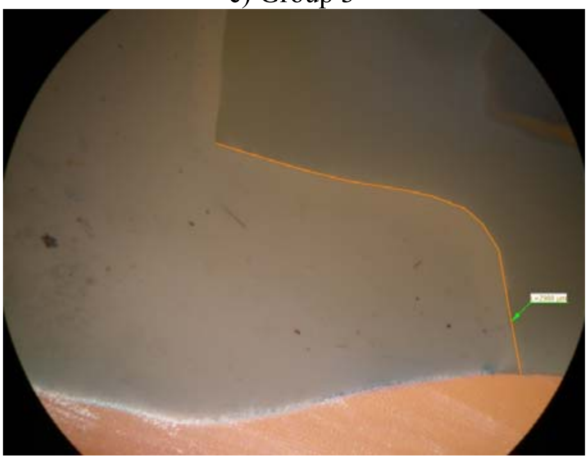
b) Group 2



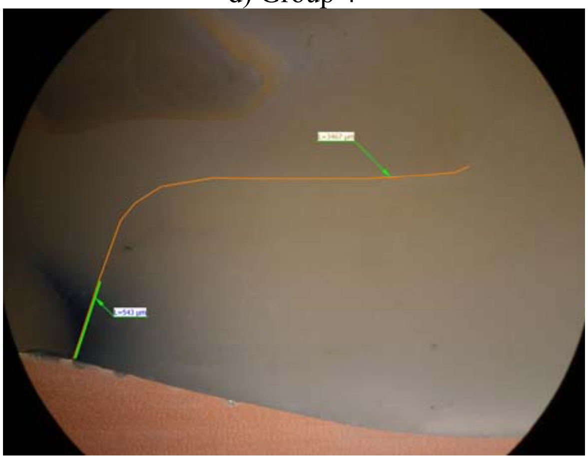
c) Group 3



d) Group 4



e) Group 5



f) Group 6

Fig.2. Evaluation of the microleakage at the interface between dentin and Gradia (a,c,d) and Barodent (b,e,f) inlays (40x magnification)

A comparative statistical analysis between the groups of specimens restored with the same composite material was performed using the Mann-Whitney test. The mean values (Mean) and standard deviations (SD) for the microleakage length and proportion were calculated (Table 2).

Table 2. Statistical data for the microleakage in dentin for the investigated specimen groups

Group	Microleakage length (µm)		Proportion of microleakage	
	Mean	SD	Mean	SD
G1	2190.85	2124.73	0.36	0.35
G2	2265.95	1943.15	0.40	0.35
G3	448.10	817.72	0.08	0.14
G4	179.80	218.40	0.03	0.03
G5	563.10	883.19	0.09	0.15
G6	480.20	843.53	0.08	0.14

For the microleakage length, statistical significant differences were observed between the Gradia specimen groups G1 and G3 (p=0.0001) and between G1 and G4 (p=0.00001), respectively. In consequence, a higher microleakage proportion was observed for G1 in comparison with G3 (p=0.0001) and in comparison with G4 (p= 0.000002). No statistical differences were observed between specimen groups G3 and G4 (p>0.05) luted with the conventional resin cement.

The results for the specimens in groups G2,G5, G6 restored with Barodent composite inlays in combination with different resin cements and adhesive systems, showed significant higher microleakage length in dentin for group of specimen G2 in comparison with group G5 (p=0.001) and also in comparison with the specimens from group G6 (p=0.0001). Statistical significant differences were observed regarding the proportion of microleakage in dentin. Specimens from group G2 had higher proportion of marginal microleakage in dentin in comparison with groups G5 (p=0.001) and G6 (p= 0.0002). The specimens in groups G5 and G6 had similar values regarding the marginal microleakage in dentin (p>0.05).

The statistical results referring to the microleakage proportion in dentin as well as in enamel for all the specimens from the groups G1-G6 are depicted in Figure 3.

4. Discussion

The clinical success of indirect restorations (inlays) depends on the technique and material used for the luting procedure using resin cements. The bonding capacity of adhesive luting agents is influenced by many factors like composition, curing mode, protocol of application or the type of the adherent surfaces i.e. enamel, dentin or composite [22].

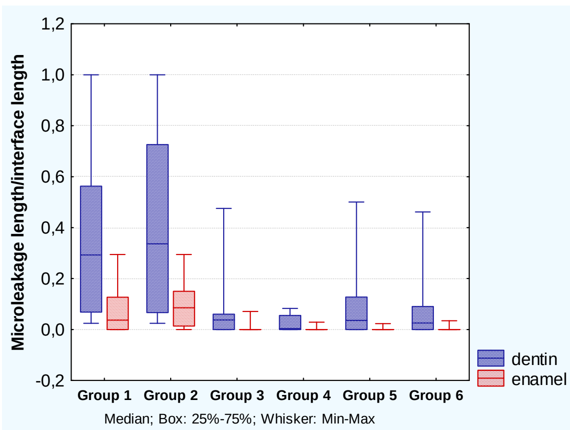


Fig.3 Box-and-whisker plots illustrating the microleakage proportion in dentin and enamel for all the specimen groups

Regarding their interaction mechanism with the dental structures, the resin cements can be classified in: a) conventional, total-etch resin cements that associate a two-or three step etch& rinse adhesive systems; b) self-etching systems that use an acidic primer, eliminating the rinsing step; and c) self-adhesive systems that have the ability to adhere to the dental structures without prior application of an adhesive system [23].

The investigation of the marginal adaptation of restorations in dentin is very important, because, in the majority of clinical cases, the proximal caries are located at this level, i.e below the cemento- enamel junction (CEJ).

The physical properties of dentin determine certain particularities of adhesion at this level. There are two processes involved in restoration bonding to dentin that is the removal of mineral phase from the dentin substrate and the filling of the remaining voids with adhesive resin that will be polymerised *in situ*, forming the so called hybrid layer. This one should provide a continuous and stable link between the adhesive and the dentin substrate. The hybrid layer is considered by many authors as the weak link in adhesive/dentin bond [17, 24].

The present study investigates the marginal adaptation of composite inlays for the restorations of cavities with the cervical limit located in dentin at 1 mm below CEJ; the luting procedure was performed with different resin cements and adhesive systems.

A dual cure self- adhesive resin cement was used in comparison with a conventional one that was associated with two- or three-step etch& rinse adhesive systems. The most uniform and homogenous interface between restorations and dentin biostructure was observed in specimens luted with the conventional resin cement Variolink II associated with two- or three-step etch& rinse adhesive systems. The microleakage tests confirmed this result. The specimens from groups G3- G6 had lower marginal microleakage values in dentin, in comparison with the specimens groups G1 and G2 that used the G-Cem self-adhesive cement. This observation is in agreement with other reported results illustrating the superiority of the conventional resin cements over the self-adhesive cements [25,26].

One can mention that, the self-adhesive resin cements were introduced to simplify the application protocol. They are less technique sensitive and they eliminate the conditioning step, assuming that the self-etching primers or functional monomers demineralise and infiltrate the dentin forming the hybrid layer. But according to the literature, they don't create a bond as strong as the conventional resin cements [27-29]. This could explain the above mentioned result.

The groups that used the conventional resin cement (Variolink II) in combination with the two-step etch& rinse adhesive (Excite DSC), formed a continuous adhesive interface, with a similar proportion of microleakage in dentin (G3 = 0.08±0.14; G5= 0.09±0.15). For the groups that used the resin cement Variolink II in combination with a three-step etch &rinse adhesive, (Optibond FL) the interface formed with the dentin structure was also continuous and uniform and the proportion of microleakage was lower (G4 = 0.03±0.03; G6 = 0.08±0.14).

There are no statistical significant differences between the microleakage length and proportion between the groups that used the same adhesive/ conventional resin cement. In these cases, the quality of resin-dentin interface was good, as illustrated by SEM investigation. These results could be explained by the properties of the adhesive systems associated to the conventional resin cement, that is the two-step etch&rinse adhesive (Excite DSC) and a three-step etch&rinse adhesive system (Optibond FL), respectively. These adhesives have in composition some inorganic filler particles that act as stress absorbent during polymerisation and consequently, they increase the adhesive sealing abilities [30, 31]. Our results are in agreement with the literature data referring to the same adhesive systems [32,33].

In spite of the fact that the two composite materials investigated in this study, i.e. the trade-mark Gradia and the experimental material Barodent have different compositions and polymerisation protocols, similar results regarding the quality of marginal adaptation of restoration were obtained. This suggests that the resin cements used for restorations luting procedure is responsible for the obtained results. Because the resin cement creates the link between the dental biostructure and the composite inlays, their ability to seal this interface is crucial for the longevity of indirect restorations.

5. Conclusions

The scanning electron microscopy and the optical microscopy coupled with the microleakage investigations, followed by a statistical analysis of data, allowed the qualitative and quantitative evaluation of the interface between composite biomaterials and tooth biostructure, as influenced by the restoring materials and the resin cements.

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.

Our results underline that the quality of the marginal adaptation and seal of composite inlays is influenced by the resin cement used for their cementation.

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