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IN VITRO ANALYSIS OF MARGINAL ADAPTATION AND RESISTANCE OF DIFFERENT DENTAL COMPOSITES: STEREO AND SCANNING ELECTRON MICROSCOPIC EVALUATION

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To compare the performance, by scanning electron microscopic analysis, of the interface between tooth and four commercial restorative composite resins in Class I cavities following exposure to acidified artificial solution, pH 4.5, with a background electrolyte composition similar to saliva, 600 teeth were divided into 4 groups. The first group was treated with microfilled light-cured Heliomolar; the second group with Durafill; the third group with the microfilled self-cured Isomolar; and the fourth group was treated using the hybrid self-cured Miradapt. All teeth of each group were randomly divided into two sub-groups: A) a control group that was immersed in artificial saliva (pH 7); B) a study group that was immersed in artificial saliva acidified with phosphoric acid (pH 4.5) in order to obtain artificial caries. The samples were examined by scanning electron microscopy. Data were analyzed using Pearson's *Chi*-squared test (χ^2) with R statistical software. The statistical analyses demonstrated significant differences in the two sub-groups A and B when considered for the light-cured composites whereas no difference was monitored for self-cured composites. Statistical analysis ($p< 0.001$) also demonstrated that the type of composite strongly influenced the infiltration grade. Our results demonstrate that incremental layering techniques might improve the marginal adaptation for light-cured composites, while self-cured show a marked polymerization contraction which can cause marginal breakdown.

Dental restorative composites are complex materials consisting of a resin matrix (usually methacrylate monomers), fillers, pigments, an initiator system, and stabilizers such as inhibitors, anti-oxidants, and UV-stabilizers.

Fillers can be divided into groups based on the size of the filler particles (composite). The tendency has been to reduce the size of the particle and to increase the filler loading in the dental composites. The first generation of dental composites mainly contained quartz particles. Currently there is a great variety in the composition of the fillers which may comprehend ceramic, glass, or quartz.

The clinical behaviour of restorative resins *e.g.,* discoloration or lack of wear resistance, varies according to the brand (1, 2). Part of this variation

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is associated to the filler, and another part to differences in the polymer matrix. Regarding the latter, the strength property of restorative resins was found to depend on the composition of the monomer (Bis-GMA) (3) and on the type and amount of functional groups involved in the polymerization reaction (4). Variations in the composition of the composite materials, as well as different degrees of conversion following polymerization, have been also observed.

The present study was designed to analyze the response of four commercial restorative composite resin materials following exposure to acidified artificial solution, pH 4.5, with a background electrolyte composition similar to saliva. The final aim was to further our understanding of the clinical performance of the four restorative composite resins under study.

MATERIALS AND METHODS

Tooth selection and preparation

Six hundred human caries-free molars, which had been extracted due to periodontal reasons, from subjects aged 40-60 years were used for this study, following informed consent. As a first step, a box-shaped cavity of 4 mm depth perpendicular to the occlusal surface was prepared by using cylindrical diamonds burs with cavosurface margins within enamel, etched with 37% orthophosphoric acid for 50 sec, washed with air-water spray, and air-dried. Afterwards, teeth were filled utilizing different dental composites and adhesive according to the manufacturer's instructions. After light curing of the adhesive, the composite was incrementally placed in two steps, 2 mm horizontal increments (Fig. 1A). For the light-cured composites, each increment was separately light-cured for 60s (Optilux 400, Kerr) while, for the self-cured ones, they were freshly mixed for each increment. The samples were prepared and restored by a single operator.

Four commercial dental composites were evaluated and the teeth under study were divided into 4 groups. The first group comprehended 200 teeth which were treated with Heliomolar light-cured microfills (Ivoclar/Vivadent, Schaan, Lichtenstein); the second group was formed by 200 teeth that were treated with Durafill (Heraeus-Kulzer, Dormagen, Germany); the third group formed by 100 teeth was treated with Isomolar self-cured microfills (Ivoclar/Vivadent Vivadent, Schaan, Liechtenstein); and the fourth group included 100 teeth that were treated using the Miradapt self-cured hybrid (Johnson & Johnson, East Windsor, NJ, USA).

Sample preparation

All teeth of each group were randomly divided into two sub-groups: A) a control one that was immersed in artificial saliva (pH 7) and stored at 37°C for 48 h; B) a study group that was immersed in artificial saliva acidified with phosphoric acid (pH 4.5) and stored at 37°C for 48 h in order to obtain artificial caries. The solution was changed after 24 h to ensure that the conditions remained constant. The tooth roots were covered with paraffin in order to prevent infiltration through the apex. The teeth were then progressively dehydrated in ethanol, cleared with xylene, and embedded in methylmethacrylate. Following polymerization, the blocks were sectioned (100-μm thick sections) with a slow-speed diamond disk saw (Gilling–Hamco, Hamco Machines Inc., Rochester, NY, USA) under copious water cooling along the tooth buccolingual longitudinal axis.

Scanning electron microscopy

In order to observe the morphology and the depth of marginal degradation as well as the demineralised enamel surface, samples were dried, sputter-coated with gold and examined in a Philips 500 SEM operating at an accelerating voltage of 25 kV.

Infiltration

Depth of marginal degradation was classified in four stages: 0, no infiltration; 1, infiltration < 2 μm; 2, infiltration >2 μm; 3, total infiltration.

Statistical analyses

Data were analyzed using Pearson's *Chi*-squared test (χ^2) with R statistical software (The R Development Core Team, Boston, MA, USA, ver. 2.4.1).

RESULTS

Table I illustrates the commercial characteristics of composites, and the percentage and distribution of infiltration of the study and control groups, plus statistical data analyses. The statistical analyses demonstrate significant differences in the two sub-groups, i.e., A (control group) and B (study group), when considered for the light-cured composites (Durafill and Heliomola,) whereas no difference was monitored for self-cured composites (Miradapt and Isomolar,). Statistical analysis ($p< 0.001$) also demonstrates that the type of composite strongly influence the infiltration grade.

Heliomolar presented the smallest infiltration rate. In fact, only 30% of the teeth filled by heliomolar

Table I. *Overview of experimental groups and results: products, manufacturers, numbers of teeth, composite type, marginal infiltration and statistical analysis for the four composites.*

	Manufacturer	Type (Formulation/ type of initiation)		N° teeth	Infiltration	Acidified saliva	Control	*P*
Durafill VS	Heraeus-Kulzer	Microfill	Light	200	0	40 (40%)	60 (60%)	0.03
					1	38 (38%)	28 (28%)	
					2	16 (16%)	10 (10%)	
					3	6 (6%)	2 (2%)	
Total						100(100%)	100(100%)	
Heliomolar	Ivoclar Vivadent	Microfill	Light	200	0	70 (70%)	70 (70%)	< 0.001
					1	15 (15%)	30 (30%)	
					2	15 (15%)	0 (0%)	
					3	0 (0%)	0 (0%)	
Total						100(100%)	100(100%)	
Miradapt	Johnson&Johnson	Hybrid	Self	100	0	18 (36%)	24 (48%)	0.50 (NS)
					1	15 (30%)	14 (28%)	
					2	12 (24%)	10 (20%)	
					3	5 (10%)	2 (4%)	
Total						50(100%)	50(100%)	
Isomolar	Ivoclar Vivadent	Microfill	Self	100	0	32 (64%)	33 (66%)	0.06 (NS)
					1	11 (22%)	16 (32%)	
					2	7 (14%)	1 (2%)	
					3	0 (0%)	0 (0%)	
Total						50(100%)	50(100%)	

P-values are based on Pearson's Chi-squared test (χ^2)
NS: not significant

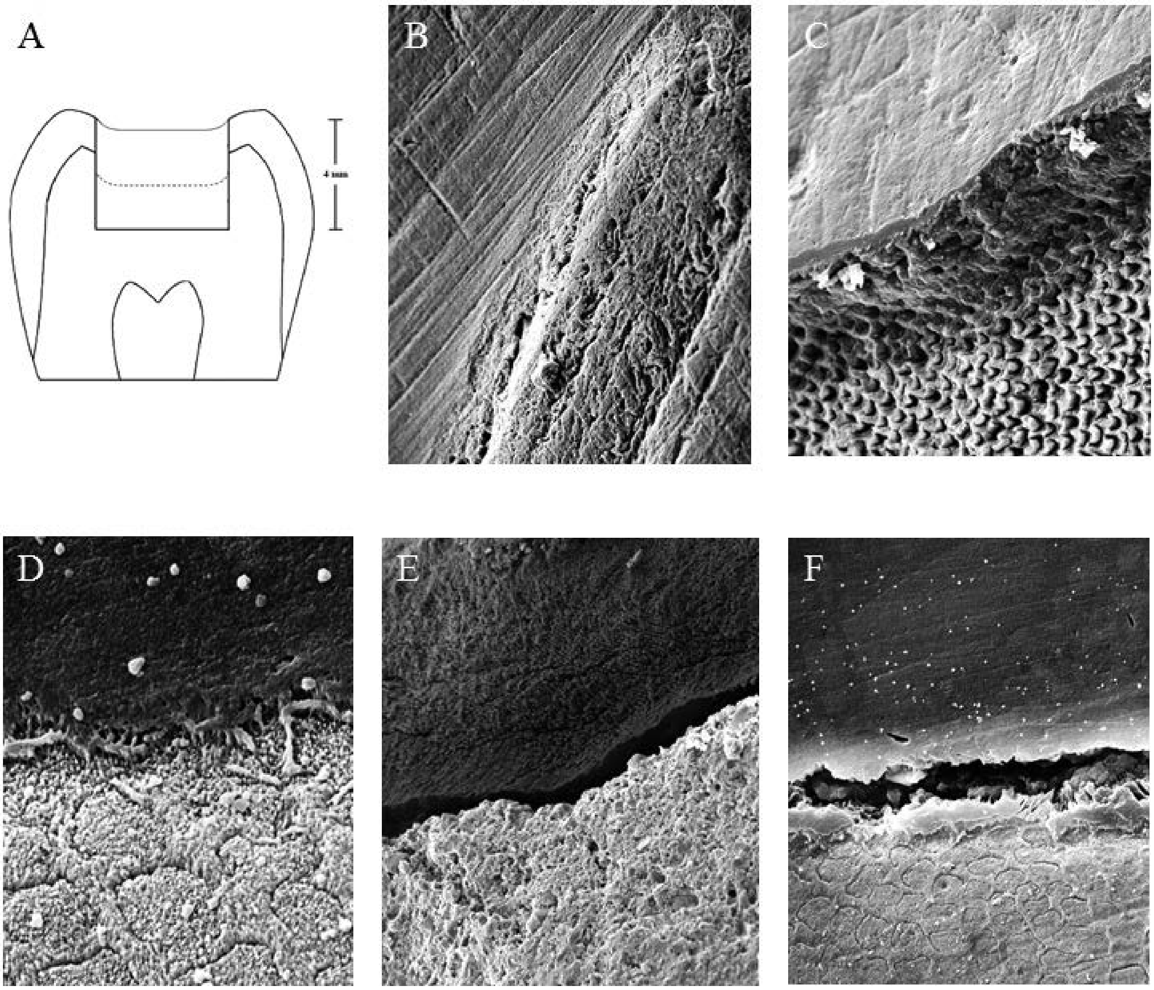

Fig. 1. *A) Schematic diagram of the filling technique.* ***B)*** *representative SEM image of a group A tooth filled by Durafill (Heraeus-Kulzer). This material exhibited a perfect marginal adaptation between enamel and composite. The enamel was intact and a thin adhesive layer between the enamel and the composite was evident. (Magnification 240x).* ***C)*** *SEM picture showing an enamel porous surface without involvement of composite-enamel interface which appeared intact (Durafill, Heraeus-Kulzer). A little step between the enamel and filling material was also evident. (Magnification 120x).* ***D)*** *Heliomolar filled tooth showed an approximate 3-5 mm thick hybrid layer with short tag-like resin extension. The artificial caries did not affect the enamel-composite margins. (Magnification 1000x).* ***E)*** *SEM image of a microfill, Miradapt, in the marginal region showing a deep breakdown between composite and enamel after artificial caries. (Magnification 120x).* ***F)*** *Typical image observed in self-cured composites: adhesive fractures occurred inside adhesive layer which appeared intact on both composite and enamel surface. SEM picture of a tooth filled with Isomolar (Ivoclar Vivadent). (Magnification 240x).*

appeared infiltrated in both A and B sub-groups. The presence of artificial caries condition was associated to a deeper infiltration. 15% of group B showed an infiltration > 2 mm.

In the Durafill filled teeth, a higher infiltration rate was exhibited by the A and B sub-groups. In both sub-groups, only a few teeth showed a total infiltration (Table I).

Miradapt and Isomolar presented the highest rate of infiltration ($p < 0.001$). However, the lack of statistical significance between the control and the study group ($p=0.5$ for Miradapt, $p=0.066$ for

Isomolar) seems to indicate that their infiltration is independent of the artificial caries procedures.

In A and B sub-groups, SEM analysis of resin-dentin interfaces highlighted a well-formed hybrid layer. An approximately 3-5 mm thick hybrid layer with short tag-like resin extension was found in all of the tested materials (Fig. 1 B, C, D). All of the analysed teeth exhibited satisfactory adaptation to enamel margins (Fig. 1 B). In sub-group B the enamel surface usually presented a porous surface with mostly an inter-prismatic loss of minerals. Additionally, a little step of few microns (2-3 μm) was created by the artificial caries process (Fig. 1C). These findings usually did not affect negatively the resistance of filling margins (Fig. 1D).

In the cases of de-bonding, the openings were usually located at the most external area of the cavity along the resin-enamel interface. Generally, light-cured composites were associated to a greater resistance to secondary caries.

Careful examination of the teeth pointed out two typical modalities of failure: 1) adhesive failure occurred at the enamel surface for the light-cured composites (Fig. 1E), and 2) adhesive fractures occurred inside the adhesive layer for the self-cured composites (Fig. 1F).

DISCUSSION

Our research group has already described tooth microstructure *in vitro* by confocal laser scanning microscopy and *in vivo* by reflectance confocal microscopy (4-8).

In the present study we studied the marginal degradation of restorative materials by comparing four commercial restorative composite resin materials. The data obtained in an established *in vitro* system provide information about class I restoration that may be of help in evaluating the different types of composites. Indeed, significant differences in marginal degradation and resistance to artificial caries between classes of materials were found. It seems likely that the type of polymerization is more important than marginal degradation in determining its resistance. This feature is supported by the presence of marginal gaps also in control groups.

SEM analysis enables to investigate micro-morphologically bonding and de-bonding characteristics within the interface and may provide a more comprehensive assessment in combination with SEM marginal evaluation (9, 10). The quantitative analysis of the internal adaptation showed that the parameter "perfect margin" varied in all experimental groups from 60 to 70% for the light-cured composites or between 24 to 33% for the self-cured. In considering the stage 1 infiltration, in which the gap was < 2 mm, by increasing this percentage to 88-100% for the light-cured and 38-49% for the self-cured one.

Although many advantages in dental materials were made in past years, polymerization shrinkage of composite-based materials remains a major problem. Polymerization shrinkage results in contraction stress, as composite resins or compomers are cured in bonded cavities. The magnitude of this stress is proportional to the volume of the material cured, is influenced by the ratio of the bonded to the un-bonded surface area (C factor), the type of initiation, the application technique and the physical and mechanical properties of restorative materials (modulus of elasticity and dimensional change) (11, 12). This stress usually concentrates at the interface between the cavity walls and the restoration, and consequently competes with the bond strength (13, 14). These features might explain in part the different results obtained in this study between self- and light cured composites. Moreover, the filling technique could represent another key point in the differences between self- and light-cured composites. The immersion in the two different solutions starts shortly after the placement of the filling materials. This could be an additional reason why the self-cured materials performed worse than the light-cured ones.

Both light-cured adhesive materials under investigation (Durafill, Heraeus-Kulzer and Heliomolar, Ivoclar Vivadent) exhibited a satisfactory adaptation to enamel margins and resistance after the artificial caries process. The analysis was statistically significant only for the light-cured composites.

Among the A study sub-group, the light-cured microfill Heliomolar (Ivoclar Vivadent) exhibited the best marginal adaptation (70% of perfect margin) and resistance to artificial caries, followed by the Durafill (Heraeus-Kulzer). Acid solution increased the depth of infiltration. 30% of the teeth filled with Heliomolar presented infiltration. In the control group this percentage showed a stage 1 infiltration while in the study group we found 15% of stage 1 and 2 infiltration, respectively. These differences were statistically significant ($p<0.001$). The self-cured materials showed significantly higher amounts of marginal opening compared to the experimental groups ($p<0.001$). A negligible portion gap formation was seen at the inner interface. Although gap-free sections were observed in some specimens, it appeared clear that the adhesion failed to withstand the detaching forces, in most cases independently of the artificial caries process ($p=0.5$ and $p=0.06$).

Following rinsing, the enamel smear layer was successfully removed and a porous layer was created in order to improve adhesion. After polymerization, the resin tags interlocked with the acid-etched enamel surfaces and formed a resin enamel hybrid layer (15).

The analysis of the enamel-resin interface by SEM revealed that, when present, failures of the interface could be observed near the enamel surface. This feature is in contrast with previous data in which the failure was reported to be generally located near the enamel-dentin junction (16). It is only possible to speculate on a differential effect of mechanical loading on the different restoration types investigated in this study. A careful examination showed two typical modalities of failure and they appeared to be strictly related to the methods of curing. Self-cured composites (Miradapt and Isomolar) usually showed adhesive fractures occurring inside the hybrid layer, in contrast with the light-cured restorative materials which showed an adhesive failure occurring at the enamel surface. This result should be ascribed to the properties of the restorative materials, since other experimental conditions, such as the C-factor of the experimental cavities, the application techniques and the bonding characteristics of the cavity, were identical in all restorations. The dimensional stability of the tooth/restorative interface is challenged from the very beginning of the polymerization reaction (17, 18). Successful initial adaptation depends on bonding systems capable of preventing separation of the restorative materials from the cavity walls. However, hindering the shrinkage establishes contractile forces in the restoration, which depends on substrate variables such as cavity configuration (19).

All composite restorations were placed in two successive increments in order to improve marginal adaptation. Incremental layering techniques are widely recognised as major factors to reduce polymerization shrinkage stress and to achieve better adaptation (19, 20). Against this theory, Versluis et al. (18) reported that theoretically bulk fillings generate less volumetric shrinkage within identical cavity.

Our results demonstrate that incremental layering techniques might improve the marginal adaptation only for light-cured composites, while self-cured ones show a marked polymerization contraction which can cause marginal breakdown.

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