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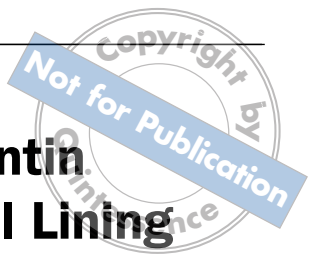
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In Vitro Microleakage of Biodentine as a Dentin Substitute Compared to Fuji II LC in Cervical Lining Restorations

Anne Raskin^a / Geoffroy Eschrich^b / Jacques Dejou^c / Imad About^d

Purpose: 1) To evaluate the marginal sealing efficacy of Biodentine at the cervical margins of approximal cavities placed in molars; 2) to evaluate and compare the use of Biodentine in combination with resin-based adhesives and a resin composite, compared with a resin-modified glass-ionomer cement (Fuji II LC).

Materials and Methods: Sixty approximal cavities were prepared on mesial and distal surfaces of 30 extracted human third molars. The teeth were randomly assigned into 6 groups of 10 cavities each: (G1) Biodentine, (G2) Fuji II LC as a filling material, (G3) Biodentine as a base + Optibond Solo Plus + silane + Filtek Z250, (G4) as in G3 without silane, (G5) Biodentine as a base + Septobond SE + Filtek Z250, (G6) Fuji II LC as a base + Optibond Solo Plus + Filtek Z250. The materials were applied according to the manufacturers' instructions. Biodentine required no dentin or enamel surface conditioning treatment. The teeth were thermocycled 2500x (5°C to 55°C). The specimens were then sealed with a 1-mm window around the marginal interface. Samples were immersed in a 50% w/v silver nitrate solution and exposed to a photodeveloping solution. The teeth were embedded in resin (Sody 33) and sectioned through the restorations. The silver penetration was directly measured using a light microscope. The results were expressed as ordinal scores from 0 to 3 at cervical, interfacial, and enamel margins. The data were analyzed with the nonparametric Kruskal-Wallis, Games Howell, and Wilcoxon signed rank tests ($p < 0.05$).

Results: No statistically significant differences were found between the 6 groups, neither for the dentin cervical margins nor for cervical lining (Biodentine or Fuji II LC)/resin composite interfaces. Statistically significant differences were observed between G5 (median score = 2.0) and the other groups (median score = 1.0) for the enamel margins. Statistically significant differences were found between enamel and dentin cervical margins in G2 (enamel median score = 1.0; dentin median score = 1.5) and G5 (enamel median score = 2.0; dentin median score = 1.0).

Conclusion: Within the limits of this in vitro study, Biodentine as dentin substitute in cervical lining restorations or as a restorative material in approximal cavities when the cervical extent is under the CEJ seems to perform well without any conditioning treatment. However, the operating time is longer than when a RMGIC (Fuji II LC) is used.

Keywords: Ca_3SiO_5 -based dentin substitute, resin-modified glass-ionomer cement, microleakage.

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A new Ca_3SiO_5 -based cement, Biodentine (Septodont; St-Maur, France), has been developed to avoid the shortcomings of direct esthetic posterior restorative materials such as operator dependence, the need for a bonding system, and poor biocompatibility. This new material was designed as a dentin substitute in cervical lining restorations and for direct posterior restorations.²⁰ It is a two-component material (powder-liquid). The solid powder part includes Ca_3SiO_5 (> 80%), CaCO_3 , and ZrO_2 . The liquid part is an aqueous solution of CaCl_2 (containing a partially modified polycarboxylate as a super-plasticizing agent)¹⁰ to reduce the water content.

Until now, resin composite based on dimethacrylate monomers (eg, bis-GMA, UDMA) could be considered as a reference material, due to its esthetic quality, relatively simple application technique, and enhanced mechanical

strength. Nevertheless, its polymerization shrinkage does not lead to perfect marginal sealing, and it results in microleakage at the interface of resin and dental tissues. The linear shrinkage of resin composites containing different amounts of dimethacrylate monomers ranges from less than 1 vol%³⁸ to more or less 5.3 vol% (pure bis-GMA).³⁶

Direct approximal restorations with resin composite are commonly carried out in daily practice, as they provide good esthetic results at low cost. However, they are known to show more leakage at enamel¹⁴ and dentin margins¹⁵ than indirect restorations. Several factors account for marginal microleakage in such situations, for instance, poor enamel quality or its absence, as well as polymerization difficulties.

These problems led to the development of cervical lining restorations.³⁹ Glass-ionomer cements (GIC) or resin-modified glass-ionomer cements (RMGIC) placed between the dentin gingival margins and the occlusal resin composite restoration are generally used for this type of application.^{1,22,34}

Indeed, GIC and RMGIC have two interesting features in such restorations: bonding spontaneously to dentin and fluoride release.²⁷ These cervical lining restorations are less technique-sensitive than resin composite restorations⁵ and show a high percentage of gap-free interfacial adaptation to dentin.⁴ These materials, with lower moduli of elasticity, can act as an elastic buffer or a stress-breaking barrier between the tooth and the resin composite, which relieves contraction and mechanical stresses.³³ However, despite the satisfactory clinical results, RMGIC has poor mechanical properties, and noticeable hydrolysis was reported after six years.¹ Furthermore, their marginal characteristics, surface properties, and color stability may cause problems.³¹

Mineral trioxide aggregate (MTA) is an example of water-based cement that has been introduced in dentistry. Nevertheless, the mechanical properties and setting time of this material (2.75 h) is not compatible with clinical use as a restorative material.¹⁸ Another mineral material has been proposed as a restorative material – Doxadent (Doxa; Uppsala, Sweden), a calcium aluminate cement – but its setting expansion seems to yield a high frequency of enamel fractures at the margins.³²

Biodentine is a new biocompatible, bioactive material which may stimulate dentin regeneration by inducing odontoblast differentiation from pulp progenitor cells.²⁰ According to the manufacturer, its setting characteristics and mechanical behavior make it suitable as a dentin substitute for direct posterior restorations. Due to its dimensional stability during setting, reliable marginal sealing of restorations made with this material may presumably be obtained.

Microleakage is defined as the clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material.¹⁹ It is one of the main reasons for restoration failures as it also induces sensitivity.² It leads to the colonization of marginal openings by microorganisms and may lead to recurrent caries and pulpal diseases. Therefore, the sealing efficacy of materials used to restore teeth is considered to be one of the

main factors affecting the longevity of restorations. Furthermore, when the tooth/restoration interface remains intact, stresses induced by the shrinking resin composite may cause microcracking of the material or tooth.³³

Two directions in research have been taken to improve the quality of marginal sealing and reduce the stress: (1) the development of adhesive systems to reduce or eliminate marginal gaps, and (2) the development of resin composites with low polymerization shrinkage. To date, no restorative system has been able to completely prevent microleakage at the dentin/resin composite restoration interface,^{12,25} and no reliable “shrinkage-free” resin composite has appeared on the market.³⁰

Numerous in vitro studies have been conducted over many years to detect and evaluate the sealing ability of restorative materials at cavity walls, but no direct correlation has been established between leakage measured with in vitro tests and long-term survival of restorations evaluated in clinical trials. This may be due to the waiting time necessary for the onset of clinical signs induced by the microleakage. Nevertheless, in vitro evaluations are needed as first screening tests for these materials, which have a limited commercial life span and undergo frequent reformulation, and also to test some parameters which cannot be tested clinically. They are one of the criteria for the selection of restorative materials and techniques.

Therefore, the aim of this study was (1) to evaluate the microleakage of Biodentine approximal restorations at enamel and dentin/material interfaces vs RMGIC (Fuji II LC, GC; Tokyo, Japan) as a reference material; (2) to evaluate the microleakage of Biodentine in approximal cavity cervical lining restorations, in combination with a self-etching bonding agent or an etch-and-rinse dentin bonding agent, either with or without silane application onto Biodentine vs Fuji II LC in combination with resin composite and the etch-and-rinse dentin bonding agent.

MATERIALS AND METHODS

Tooth Selection and Preparation

Thirty extracted human maxillary and mandibular third molars, free of cracks, caries and restorations, were used in this study. The work was done in agreement with the French ethics laws. The extracted teeth were stored in an aqueous chloramine solution (1%) at 4°C for no more than 1 month.

Cavity Preparation and Restorative Procedure

The teeth were scaled and cleaned with slurry of pumice before cavity preparation. The mesial and distal surfaces of each tooth were randomly attributed to one of 6 groups (n = 10 per group) (Fig 1) by using a random numbers table for assigning teeth, and randomizing by blocks so that (1) half of the restorations of a group were made on the mesial face and other half on the distal face, and (2) every restoration of a group was placed alternately with a restoration from the 5 other groups.

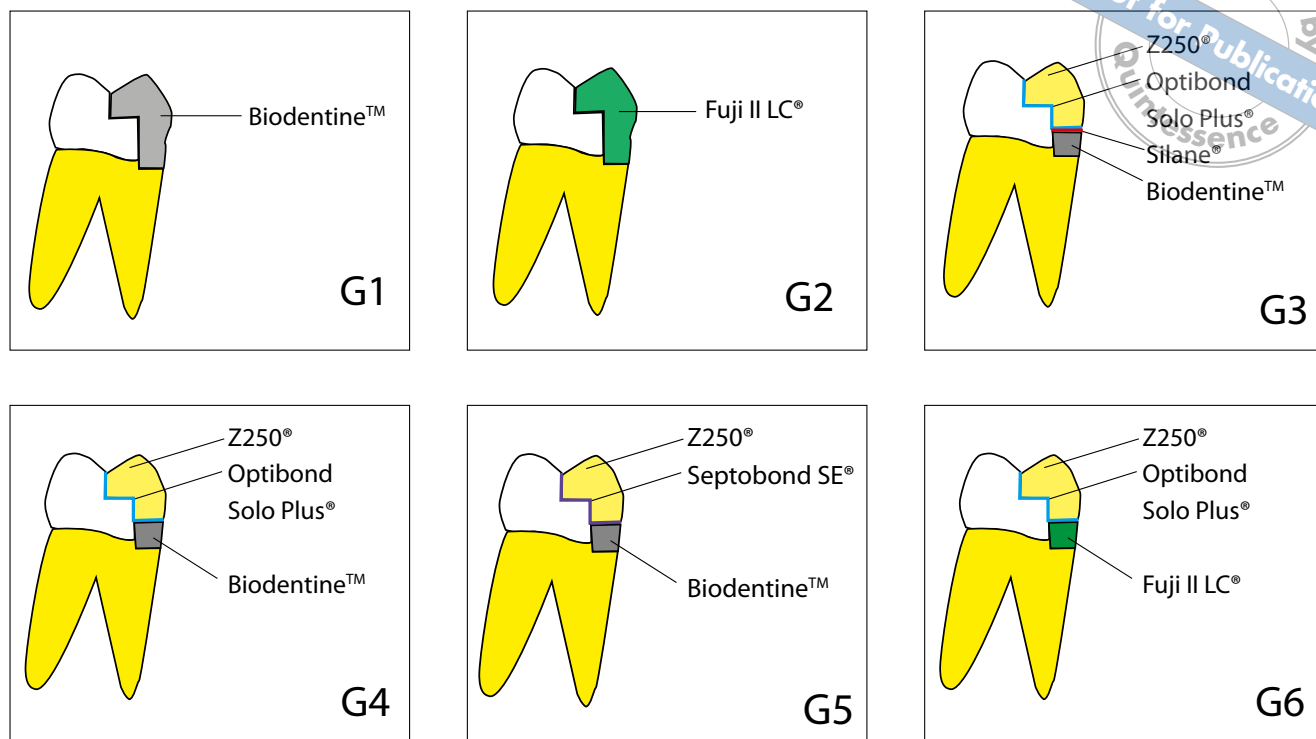


Fig 1 Class II cavities designs in the 6 experimental groups. G1: Class II filled with Biodentine; G2: Class II filled with Fuji II LC; G3: dentin substitute (Biodentine) covered with silane, Optibond Solo Plus and filled with Z250; G4: dentin substitute (Biodentine) covered with Optibond Solo Plus and filled with Z250; G5: dentin substitute (Biodentine) covered with Septobond SE and filled with Filtek Z250; G6: dentin substitute (Fuji II LC) covered with Optibond Solo Plus and filled with Filtek Z250.

Table 1 Description of the materials used in the study

Materials	Manufacturer	Components
Biodentine (BN: 47325)	Septodont; St Maur Des Fosses, Val-de-Marne, France	Solid: Ca_3SiO_5 (> 80%), CaCO_3 , ZrO_2 Liquid: Water, CaCl_2 , partially modified polycarboxylate
Fuji II LC (BN: 0710021)	GC; Tokyo, Japan	Liquid: polyacrylic acid Powder: Al_2O_3 - SiO_2 - CaF_2 glass and HEMA urethane dimethacrylate
Silane Porcelain Primer (BN: 594927)	Bisco; Schaumburg, IL, USA	Single component pre-hydrolyzed no-mix silane primer, acetone, ethanol
Optibond Solo Plus (BN: 2890041)	Kerr; Orange, CA, USA	Ethanol, bis-GMA, HEMA, GPDM, silica, barium glass, sodium hexafluoro-silicate, camphorquinone
Septobond SE (BN: 37076)	Septodont	Primer : HEMA, polymerization agent, water, ethanol Bonding : urethane methacrylate, photo-initiator, ethanol
HEMA: 2-hydroxyethylmethacrylate, GPDM: glycerophosphoric acid dimethacrylate, BN: batch number.		

Cavity preparations were made using cylindrical diamond burs (Diatech, Heerbrugg, Switzerland, ISO-NO 806 314 173 524 031 10.0) under a water-cooled high-speed handpiece. Two standardized approximal cavities were prepared at the cemento-enamel junction (CEJ) on the mesial and distal surfaces of each tooth. The enamel and gingival margin in dentin/cementum was prepared to a butt joint. Cavity dimensions are described in Fig 2.

When Biodentine or Fuji II LC were used as cervical liner, the same resin composite was used for all resto-

rations (Filtek Z250, shade A3, 3M ESPE; St Paul, MN, USA). All materials (Table 1) were handled and applied according to the manufacturers' instructions. All restorative procedures were performed by two skilled operators and all restorations of each experimental group were randomized between the two operators, so that each operator carried out half of the restorations of each group and in each group half of the mesial and half of the distal restorations. The six groups are defined in the following.

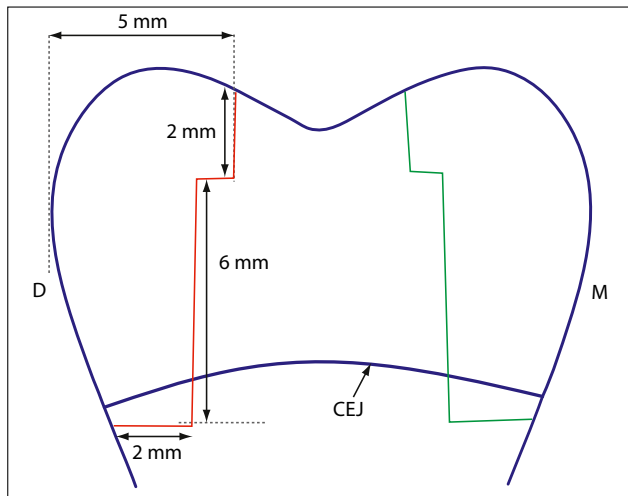


Fig 2 Class II cavities designs and dimensions.

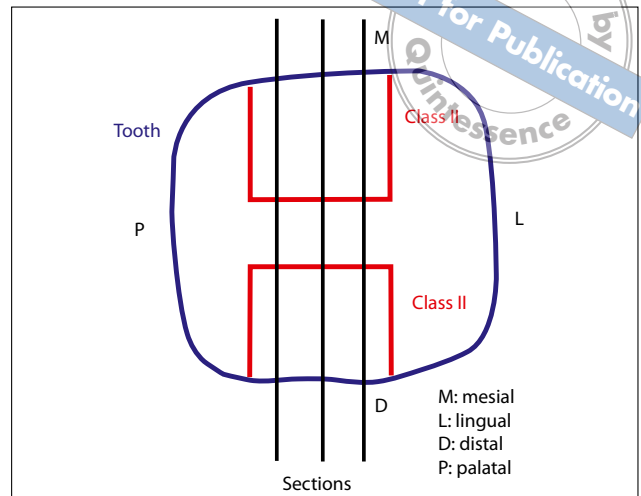


Fig 3 Orientation and position of the three sections per restoration (occlusal face).

G1 (Biodentine)

After mixing 1 g of powder and 180 µl of liquid in a capsule for 25 s with an amalgam mixing machine at room temperature (23 ± 1°C), a matrix (Omnimatrix, Ultra-dent; South Jordan, UT, USA) was used and the cavities were filled with a cement spatula and amalgam pluggers without any enamel or dentin surface treatment.

The teeth were then stored in an incubator (37°C and 90% relative humidity) for 60 min before the finishing and polishing procedures were carried out with a contouring diamond bur (Maillefer; Montigny Le Bretonneux, France, ISO 806 314 249 514 010) under water spray.

G2 (Fuji II LC)

GC Dentin Conditioner (10% polyacrylic acid solution) was applied to the surfaces for 20 s and rinsed thoroughly (10 s) with water, after which the cavities were filled with Fuji II LC. The teeth were then stored in an incubator (37°C and 90% relative humidity) for 60 min before the finishing and polishing procedures, performed with a contouring diamond bur (Maillefer) under water spray.

G3, G4 and G5

Cavities were filled as described for G1. The Biodentine was then removed down to the level just below the maximum convexity of the proximal aspect (approximately 2 mm).

G3 (Biodentine, silane, Optibond Solo Plus): 37% phosphoric acid (3M ESPE) gel was applied for 30 s and 15 s to enamel and residual dentin, respectively, before being thoroughly rinsed (10 s) and gently dried for 3 s. The silane porcelain primer (Bisco; Schaumburg, IL, USA) was applied onto the surface of Biodentine and thoroughly dried for 30 s. Optibond solo Plus (Kerr; Orange, CA, USA) was applied with a microbrush on all surfaces (dentin, enamel, and Biodentine), and light cured for 20 s.

G4 (Biodentine, Optibond Solo Plus): The procedure was the same as for G3 but the silane was not applied to the Biodentine before the adhesive procedure.

G5 (Biodentine, Septobond SE): The etching/primer was applied to all surfaces (dentin, enamel, and Biodentine) for 15 s and then air dried for 10 s. The bonding agent was also applied to all surfaces for 15 s, air dried for 15 s, and then light cured for 20 s.

The resin composite was applied in three increments to a maximum of 2 mm. Each increment was light cured for 40 s with a light-curing unit (Elipar Trilight, 3M ESPE) in standard mode (800 mW/cm²). The finishing procedure was immediately performed using a contouring diamond bur (Maillefer) with water spray.

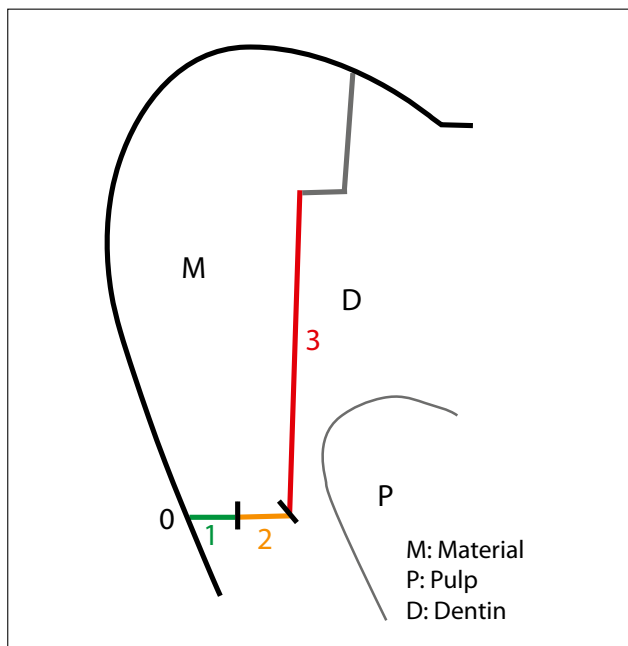


Fig 4 Tracer penetration evaluation at dentin/biomaterial interface.

Table 2 Microleakage median scores at enamel, dentin, and interface margins in the six experimental groups

Materials	Median scores		
	Enamel	Dentin	Interface dentin substitute/resin composite
G1: Biodentine	1.0ab	1.0	
G2: Fuji II LC	1.0ab	1.5	
G3: Biodentine + Silane Porcelain Primer + Optibond Solo Plus	1.0ab	1.0	1.0
G4: Biodentine + Optibond Solo Plus	1.0a	1.0	1.0
G5: Biodentine + Septobond SE	2.0b	1.0	1.0
G6: Fuji II LC + Optibond Solo Plus	1.0a	1.0	1.0
Kruskal-Wallis + Games Howell	S (0.05)	NS (p = 0.46)	NS (p = 0.43)

For enamel maximal median scores, the differences between the groups with similar letters (vertical reading of the table) are not statistically significant.

G6

Fuji II LC was applied as described for G2 and the restorative procedure with resin composite was the same as in G4 using Optibond Solo Plus.

Dye Penetration

After restorations were performed, the samples were initially stored for one day in physiological saline solution at 37°C and then thermocycled in water baths for 2500 cycles, alternatively at 5°C and 55°C with a dwell time of 15s.

To prepare for the dye penetration test, all tooth root apices were sealed with Fuji II LC to prevent the tracer penetrating through the apex. The surfaces of the teeth were coated with two layers of red nail varnish (Sech'Express, Gemey; Orléans-Ormes, France) 1 mm beyond the restoration margins. All specimens were placed in a 50 wt% weight silver nitrate aqueous solution in the dark for 2 h. They were then rinsed with water and placed in a radiographic developing solution (Ilford, ID11; Mobberley, Cheshire, UK) for 6 h under a fluorescent light. The teeth were thoroughly washed with water and acetone to remove the nail varnish and embedded in a slow-curing polyester resin (Sody 33, ESCIL; Chassieu, France). After being embedded in the slow-curing resin, the teeth were sectioned with a low-speed Isomet saw as described in Fig 3.

Three sections per restoration of approximately 500 µm thickness were made. These provided 6 surfaces for the dye penetration evaluation.

Microleakage Evaluation

Photographs were obtained for each of the sections, and dye penetration evaluations were made directly under a binocular stereomicroscope at 125X and recorded as the penetration depth along the cavity walls (ordinal scale; Fig 4). Scores for cervical margins were: 0 = no penetration; 1 = leakage extending within first 1/2 of the cavity wall; 2 = leakage extending beyond 1/2, but not

as far as the cervical cavity floor; 3 = leakage extending beyond the cervical cavity wall and reaching the cavity floor. Scores for Biodentine/RMGIC to resin composite interfaces were: 0 = no penetration; 1 = leakage extending within first 1/2 of the interface wall; 2 = leakage extending beyond 1/2 but not as far as the interface wall; 3 = leakage extending beyond the interface wall and reaching the cavity floor. For enamel margins, the scores were: 0 = no penetration; 1 = leakage extending within first 1/2 of the enamel wall; 2 = leakage extending beyond 1/2, but not as far as the dentin-enamel junction; 3 = leakage extending beyond the enamel-dentin junction.

The penetration was evaluated separately by two examiners and, where there were differences in evaluation, the surface was evaluated once more by the two examiners simultaneously to decide on the final score. The maximum score found among the 6 evaluated surfaces per restoration was selected to summarize the information, since just one channel of leakage is sufficient to affect the quality of the restoration, especially if the channel is deep.⁹

Statistical Analysis

The Kruskal-Wallis nonparametric test and the Games Howell post-hoc test were used to compare the scores found for the different adhesives systems at the enamel and dentin margins, and at the dentin substitute/resin composite interface for groups 3 to 6. For each restorative procedure, the Wilcoxon T-test was used to compare enamel and dentin microleakage. Interexaminer reliability was tested with a nonparametric Spearman correlation test. The statistical significance level was set at 0.05.

RESULTS

The interexaminer reliability was high (Rho = 0.970, p < 0.001).

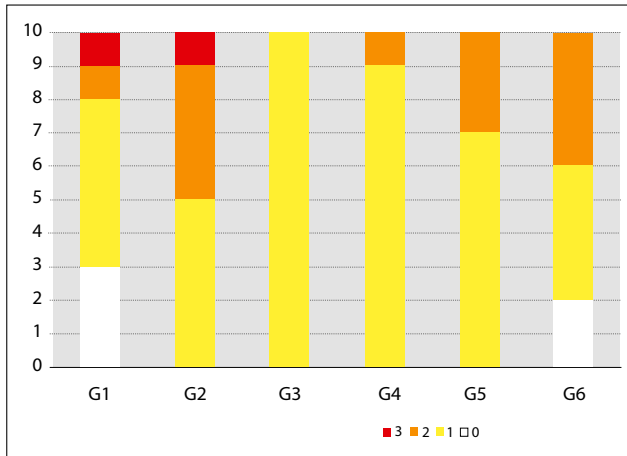
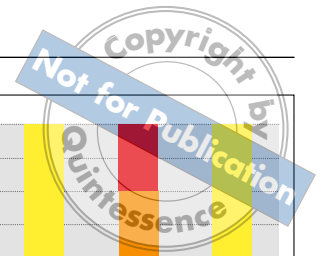


Fig 5 Frequency of dentin microleakage scores.

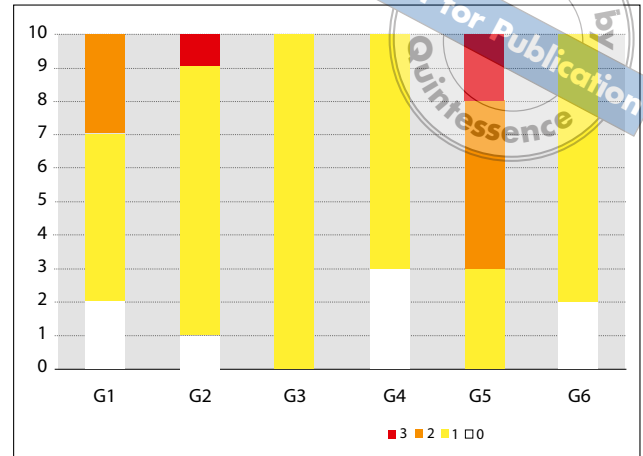


Fig 6 Frequency of enamel microleakage scores.

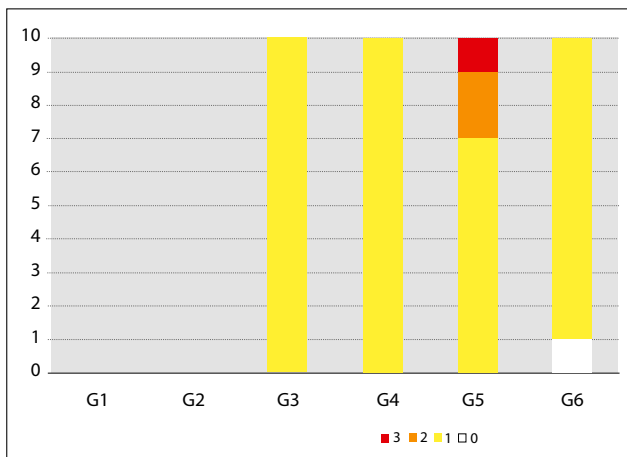


Fig 7 Frequency of interface microleakage scores.

Dye Penetration at the Dentin Cervical Margins

No statistically significant differences were evident between the 6 groups (Kruskal-Wallis, $p = 0.46$; Table 2). The median of maximum scores recorded was low (median score = 1.0) for all groups, except for G2, which was a little higher (1.5) (Table 2; Fig 5). Only G1 and G2 presented score 3. All groups, except G3, presented score 2. Only G1 and G6 presented score 0. Score 1 was predominant in all groups (Fig 5).

Dye Penetration at the Enamel/Restoration Interface

Statistically significant differences were found between the groups (Kruskal-Wallis, $p = 0.05$); Table 2). Dye penetration was higher at the enamel restoration interface of G5. The median of maximum scores recorded was also weak (1.0) for all groups, except for G5, which was higher (2.0). G2 and G5 presented score 3. G1 and G5 had score 2. G1, G2, G4, and G6 showed score 0. Score 1 was predominant in all groups, except for G5 (Fig 6).

Dye Penetration at the Dentin Substitute/Resin Composite Interface

No statistically significant differences were noted between the 4 groups (Kruskal- Wallis, $p = 0.43$; Table 2). The median of maximum scores was low (1.0) for all 4 groups (Table 2). Only G5 presented scores 2 and 3, while only G6 presented score 0. Score 1 was predominant in all groups (Fig 7).

Comparison of Enamel and Dentin Interface Microleakage

Statistically significant differences were observed between the dentin and enamel interface for G2 and G5. Dye penetration was higher at the dentin/Fuji II LC interface (G2; Wilcoxon, $p = 0.043$) and higher at the enamel/Septobond SE interface (G5; Wilcoxon, $p = 0.431$) (Figs 5 and 6).

DISCUSSION

Marginal sealing of approximal restorations with gingival margins below the CEJ with the new mineral material (Biodentine) was equivalent to that of the RMGIC (Fuji II LC) restorations. Different restorative materials can be successfully applied on top of this new material without any significant leakage at the interface between the new dentin substitute and the overlaid restorative material.

Additionally, the results of this work were obtained without any dentin conditioning treatment before Biodentine application, suggesting that the new material is suitable as a dentin substitute. Its application is simple but more time consuming, because the setting time (10 min) is longer than RMGIC's, and a second appointment is sometimes necessary to complete the restoration.

Optibond Solo Plus is a two-step etch-and-rinse adhesive system. It has been the subject of extensive studies for more than 10 years by many authors and is one of the most reliable adhesive systems in this class of materials.^{13,23,28,29,35} Septobond SE is a two-step self-etching

adhesive system. As seen in other studies,^{17,19,26} the enamel microleakage is often higher with some self-etching agents than with etch-and-rinse adhesive systems, especially if the enamel is not conditioned.

Silane Porcelain Primer was tested in order to establish whether it could decrease the microleakage when placed on the surface of Biodentine. No chemical support exists between this cement and dental adhesives, while Silane Porcelain Primer could lead to chemical connections with the mineral fraction of the cement.

Filtek Z250 is a microhybrid resin composite. Often used in experimental studies of bond strength and microleakage in adhesive restorative systems,^{3,6,7,24} it has good mechanical (flexural strength and modulus) and physical properties (volumetric polymerization shrinkage and water sorption).⁸ Whatever the surface treatment used on Biodentine, this material can be used as dentin substitute in combination with resin composite, since sealing efficacy at the Biodentine/resin composite interface did not differ from that obtained with RMGIC as a dentin substitute. It deserves mentioning that the resin composite application to Biodentine and enamel required a simple surface treatment (etching for 15 s with 37% phosphoric acid and applying a bonding agent). This resulted in a good adhesion of the resin composite on both the new mineral material and the RMGIC (Fuji II LC).

The comparison between enamel and dentin interface microleakage showed that Septobond SE had higher leakage in enamel than in dentin, while Fuji II LC showed higher leakage in dentin than in enamel. However, none of the materials completely prevented silver nitrate microleakage at the dentin-, enamel-, or dentin substitute/material interfaces.

The technique sensitivity of adhesive restorative procedures has often been discussed.¹⁶ However, in this study, all the restorations were randomized and performed by two skilled practitioners. Thus, the results obtained with the adhesive restorative materials were not likely to be influenced by the operator's skills.

Since cervical lining restorations have been proposed as an alternative to direct resin composite restorations, many clinical studies have been published to evaluate the durability of this type of restoration.^{1,21,39} The traditional cervical lining technique with a conventional GIC showed failure rates between 13% and 35% after 2 years and 75% after 6 years. With the use of RMGIC instead of conventional GIC, the GIC/resin composite interface issue has been solved by methacrylate-based resin in the two materials.

Although no surface treatment was carried out with Biodentine, nor has any specific instrumentation been developed for filling cavities and finishing margins, the marginal microleakage of restorations made with the new material was equivalent to that of a reliable adhesive restorative system and was independent of the dental tissue (enamel or dentin). One possible explanation could be the formation of hydroxyapatite crystals at the Biodentine surface. Indeed, it has been demonstrated that soaking this tricalcium cement in reconstituted saliva led to the formation of a hydroxyapatite layer on its surface.⁴⁰ Thus,

it may be assumed that this layer prevented the degradation of the cement and kept the marginal sealing intact. The tendency of RMGIC to be influenced by exposure to water before setting has been documented.⁵ This suggests that the good results obtained with Fuji II LC in this study may be due to water sorption that relieves setting shrinkage.³⁷

Under the conditions of this study, Biodentine, like Fuji II LC, can be used as a suitable dentin substitute in the same way as Fuji II LC. Used in this way, it should be covered by a resin composite; consequently, its deformation under mechanical stress may be considered as a less important factor. However, if Biodentine is to be used as a bulk temporary restoration material, this factor has to be taken into account. These *in vitro* results showed that the physical properties of Biodentine are adapted to clinical practice.¹⁰ Further research is required in this area to confirm its long term behavior.

CONCLUSION

Within the limits of this study, Biodentine may be used without a dentin conditioner as a dentin substitute in cervical approximal cavities or as a bulk provisional restoration where the cervical extent is under the CEJ. Used as a dentin substitute, Biodentine may be applied in conjunction with a dentin bonding agent. A silane placed onto Biodentine did not seem to improve the sealing.

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Clinical relevance: Although the operating time is longer than for Fuji II LC, Biodentine can be used as a dentin substitute under resin composite in cervical lining restorations or as a filling material without any conditioning treatment.