Evaluation de l'adaptation marginale de différents matériaux de remplacement de la dentine dans la technique open sandwich des restaurations de classe II in vitro

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Thèse préparée sous la direction du Professeur Ivo KREJCI et PD Dr Tissiana BORTOLOTTO

Evaluation de l'adaptation marginale de différents matériaux de remplacement de la dentine dans la technique open sandwich des restaurations de classe II *in vitro*

Thèse

présentée à la Faculté de Médecine

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par

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INTRODUCTION

De nos jours, une part importante, et toujours croissante, de la population ne parvient pas à assumer les coûts des traitements dentaires. Selon une étude récente menée dans la région de Genève, 14.5 % de la population renoncent aux traitements médicaux par manque pécuniaire, et 74 % se privent de traitements dentaires (Wolff et al. 2011). De ce fait, il est extrêmement important que la profession dentaire propose des solutions restauratrices sobres, à moindre coût, desquelles pourrait bénéficier cette partie de population. Quant à la population vieillissante, elle rencontre le même problème budgétaire, il est donc primordial d'accorder aux procédures simples et rapides une importance prédominante. Ce point reste essentiel, tout spécialement au vu du fait que la proportion de personnes âgées qui conserve une denture naturelle est en nette croissance (White et al. 2012).

Depuis plus de cent cinquante ans, l'amalgame a été considéré comme le matériel de restauration universel, avéré, et abordable pour tout à chacun, lorsqu'il s'agit de restauration des dents postérieures. Son application était estimée et approuvée facile et rapide, ses manipulations techniques simples. C'est pourquoi l'amalgame a fait partie du matériel de restauration, d'une part à but social – pour une large population démunie ou à ressources financières limitées – et d'autre part, à but sanitaire : nombre de patients souffrent de mauvaises conditions orales, comme il a d'ailleurs été souvent constaté chez les personnes âgées (Bharti et al. 2010).

Cependant, ces dix dernières années, et ce de plus en plus fréquemment, l'amalgame a été passablement critiqué pour différentes raisons comme l'esthétique, la conductivité thermique, l'expansion de prise et l'invasivité pour assurer une macro-rétention. La critique actuelle la plus récurrente envers l'amalgame traite de son composant principal, le mercure. Il est estimé hautement toxique pour l'environnement, et décrit comme tel dans la Convention Minamata, adoptée en octobre 2013, entrée en vigueur en août 2017, et ratifiée par quatre-vingt-neuf pays (Basu 2018). Cette convention s'efforce de limiter de façon drastique l'emploi du mercure, tout en ayant conscience de l'impact majeur de cette décision sur l'utilisation des amalgames en dentisterie. (https://www.fdiworlddental.org/what-we-do/advocacy/dental-amalgam).

Quand bien même l'amalgame était estimé norme d'excellence quant aux restaurations directes, la dentisterie restauratrice actuelle le délaisse pour préférer travailler avec les restaurations adhésives directes exemptes de métal, et sans mercure. Elles ne requièrent également aucune procédure de préparation de cavité - qui, finalement, reste très invasive - et demeurent une approche qui sied parfaitement bien à la philosophie actuelle de la dentisterie minimalement invasive, une option de plus en plus privilégiée par la profession dentaire (Oliveira et al 2016).

Ces restaurations directes exemptes de métal sont du domaine des dits « matériels composite ». Ces produits peuvent être polymérisés de façon chimique, par la lumière visible ou via la combinaison de ces deux mécanismes, également appelée « polymérisation duale ». Actuellement, la polymérisation par la lumière est de plus en plus répandue, ce qui oblige le dentiste à utiliser une technique de stratification pour pouvoir insérer le matériel dans la cavité afin de durcir correctement les matériaux, et ainsi dépasser la profondeur limitée de la polymérisation lumineuse.

Les composites dentaires sont constitués d'une matrice résineuse, elle-même composée de Bis-GMA (Bisphénol-A-Glycidyl méthacrylate) ou de UDMA (Uréthane diméthacrylate) comme molécules principales, lesquelles sont complétées de plus petites molécules organiques. Ces molécules organiques ajustent la consistance de la matrice résineuse, et améliorent l'adhésion lors de la polymérisation. Les charges dispersées dans la matrice résineuse jouent le rôle de renforcement mécanique. Elles réduisent la contraction de la résine lors de la polymérisation. A noter que, dans son état naturel, la résine affiche des valeurs de 6 à 7 Vol% ; elles plafonnent à environ 1 à 3 % lors de l'incorporation des particules de remplissage. Elles permettent également d'obtenir une radio-opacité du matériel de restauration suffisante au vu du fait qu'il est composé de métaux lourds. Les charges sont souvent manufacturées avec du verre, de la céramique ou des particules de quartz, elles peuvent cependant être fabriquées avec de la silice fumée, TiO_2 ou ZrO ou des particules organiques de remplissage prépolymérisées. Pour lier les particules de remplissage à la matrice organique résineuse, des silanes organiques revêtent ces particules, et les imbriquent aux molécules de la matrice organique en structures de méthacrylate. Et ce, de telle façon que se crée un matériel dentaire de restauration sans métal lequel est suffisamment résistant pour être considéré comme solution restauratrice définitive. Toutefois, il ne dispose pas de propriétés adhésives. Il a besoin d'être associé à un système adhésif externe afin d'éviter la préparation macromécanique de cavités rétentrices, mais également pour optimiser le scellement marginal.

L'idée première de se servir d'un système adhésif facilitant le lien entre produit et dent a été adoptée lorsque Buonocore a découvert, en 1955, que l'utilisation d'un acide phosphorique à 85 % améliorait la rétention mécanique d'une résine acrylique sur l'émail (Buonocore 1955). L'application d'un acide phosphorique rend la surface amélaire plus rugueuse. De ce fait, elle peut être mouillée, et pénétrée par un agent de liaison résineux à faible viscosité. Une fois l'agent de liaison polymérisé, la surface de l'émail a atteint un seuil de liaison solide grâce aux phénomènes micromécaniques (Buonocore et al. 1968). Depuis, la formation d'extension en résine dans les microrugosités interprismatiques et intercrystalliques de l'émail a été considérée mécanisme prédominant, et les composites de liaison en résine se fixent d'autant mieux à l'émail mordancé à l'acide phosphorique (Buonocore et al. 1968, Gwinnett & Matsui 1967). Ces monomères situés à l'intérieur des rugosités de la surface amélaire sont polymérisés et se scellent aux cristaux d'hydroxyapatite (Van Meerbeek et al. 2003).

Cependant, l'adhésion à la dentine représentait un plus grand défi. Quand bien même Buonocore avait déjà entamé des expériences avec les systèmes adhésifs au début des années soixante, ses efforts ne furent pas nécessairement récompensés, puisque la première génération des adhésifs dentaires a montré des forces d'adhésion très faibles, variant de 1 à 3 MPa (Söderholm et al. 2007). Ce ne fut que dans les années quatre-vingt que les premiers produits « etch-and-rinse » efficaces et les systèmes adhésifs auto-mordançant ont fait leur apparition, grâce à Fusayama et Nakabayashi qui ont fait des systèmes adhésifs la base de leur recherche (Söderholm et al. 2007). Les progrès de ces systèmes ont débouché sur la création de produits cliniques véritablement efficaces, tant dans le groupe « etch-and-rinse » (Peumans et al. 2012) que dans celui des adhésifs auto-mordançants (Peumans et al. 2015).

Le développement de systèmes adhésifs efficaces a finalement conduit à la grande et large utilisation des résines composites pour la restauration des dents postérieures, étant le matériel le plus fréquemment utilisé dans les restaurations de classe I et de classe II grâce à ses propriétés mécaniques propres à résister à la charge occlusale, à ses propriétés esthétiques, et à son habilité à se coller aux structures dentaires via les systèmes adhésifs.

Les restaurations directes en composite ont prouvé leur bon comportement clinique sur le long terme, même après plusieurs décennies (Montag et al. 2018). Cependant, placer des restaurations en composite direct via la méthode classique, avec des matériels en composite polymérisés à la lumière nécessitant une technique de stratification complexe, est considéré délicat, chronophage, et plus coûteux que de placer des restaurations en amalgame (Jackson et al. 2016). Les restaurations en composite direct postérieures classiques réalisées avec un matériel standard polymérisés à la lumière peuvent ne pas être considérées comme une option valable pour remplacer l'amalgame en dentisterie sociale, et également pour les personnes âgées. D'autres techniques et matériels de restauration ont été, de ce fait, proposés pour remplacer l'amalgame dans cette direction, tels les ciments en verre ionomère (CVI) ou les dits composites « *bulk-fill* ».

Les CVI sont un mélange de poudre de verre en silicate (verre de calciumaluminofluorosilicate) et d'acide poly-acrylique, un ionomère. De l'eau peut être ajoutée à la place de l'acide, modifiant alors les propriétés du matériel et ses indications. Les CVI libèrent du fluorure, et sont donc supposés avoir un effet protecteur contre les caries. De plus, ils bénéficient d'une certaine auto-adhésion, relativement faible certes, mais qui peut servir à la rétention de matériel dans les cavités non rétentrices (Sidhu et al. 2016). C'est pourquoi les préparations de cavité conservatrices, comme dans le cas de la technique du traitement dit « restauration atraumatique » (ART), peuvent s'élaborer avec des CVI (Frencken et al. 2010). Ces ciments sont injectés, sous forme liquide à l'aide d'une capsule de mélange, dans la cavité, en une seule couche. Leur procédure d'application est donc rapide et simple. Au départ, leurs propriétés mécaniques n'étaient pas suffisamment probantes pour qu'ils soient utilisés dans les cavités de classe II sur dents postérieures. Grâce à un développement approfondi de leur formule pour obtenir une plus haute viscosité, les verres ionomères ont, de plus en plus, été considérés comme matériels de restauration définitifs pour les dents postérieures. Toutefois, les preuves pour appuyer ces dires sont encore faibles, raison pour laquelle les options des matériels entrent en considération, tels les composites « *bulk-fill* » (Mickenautsch et al. 2015).

Les composites type « *bulk-fill* » sont des composites polymérisés à la lumière modifiés. Ils sont réputés avoir une profondeur importante d'illumination et une contraction réduite lors de la polymérisation, ce qui devrait limiter le nombre de couches de composite nécessaires pour la restauration de cavités postérieures profondes, et faire de la technique de restauration une technique nécessitant de plus simples matériels, moins délicate, plus rapide, et ainsi moins coûteuse pour le patient (Jackson 2016).

L'un des premiers matériels en composite type « *bulk-fill* » utilisé le plus fréquemment est le « Smart Dentin Replacment » (SDR) de la maison dentaire Dentsply. C'est un composite fluide contenant 68% en masse de charges. Il dispose d'une matrice monomère spéciale, laquelle est supposée avoir une contraction plus faible à la polymérisation, ceci dû à l'incorporation d'un modulateur de polymérisation au poids moléculaire élevé qui se loge de manière chimique au centre du squelette polymérisable du monomère. Le UDMA modifié breveté dispose d'un poids moléculaire plus élevé (849 g/mol) que le UDMA conventionnel (470 g/mol), le Bis-GMA (512 g/mol) et les autres monomères conventionnels. Dans une étude clinique rétrospective contrôlée sur cinq ans, ce matériel a montré une durabilité légèrement meilleure, cependant, statistiquement pas significative par rapport à un composite standard appliqué par stratification de 2 mm par couche (Van Dijken & Pallesen 2016). Le SureFil™ SDR dispose d'un module d'élasticité relativement bas et d'une contraction de polymérisation qui résulte d'un stress plus faible au niveau des parois de la cavité (Burghess & Cakir 2010), et même plus faible que celui des matériels fluides conventionnels (Illie & Hickel 2011). Le SureFil™ SDR est également un matériel de remplacement dentinaire qui peut être étalé sur 4 mm d'épaisseur sans interférence avec la pénétration de la lumière de polymérisation. Une étude récente a démontré que le SureFil™ SDR, appliqué comme substitut dentinaire de remplissage de 4 mm, est performant en termes d'intégrité marginale dans l'émail et la dentine (Matthias et al. 2011). La notion d'intégrité marginale représente la qualité de la marge des restaurations dentaire, et de l'adaptation dans ce cas du matériau de restauration directe par rapport au substrat dentaire. Une mauvaise adaptation ou un manque à ce niveau sera le lieu d'accumulation de débris alimentaires, et un potentiel site de carie secondaire. Néanmoins, vu que la dureté du SureFil™ SDR n'est pas suffisamment élevée pour supporter la charge occlusale de la restauration, il doit être recouvert d'une couche occlusale de composite de restauration standard (Ilie et al. 2013).

Cette configuration représente, de façon générale, une restauration appelée « restauration ouverte en sandwich », proposée par McLean : une couche basique, habituellement réalisée avec un CVI, remplace la dentine, puis est recouverte d'une seconde couche occlusale faite de composite de restauration (McLean 1992). Avec la technique du sandwich ouvert, utilisée pour la restauration des cavités postérieures de classe II, les deux couches de matériels de restauration appliquées sont exposées à la cavité orale au niveau des surfaces proximales, phénomène de première préoccupation quant au succès clinique sur le long terme (Koubi et al. 2009).

Le taux d'échecs cliniques de la technique du sandwich ouvert avec du verre ionomère étant situé entre 13 et 35 % après deux ans, et à 75 % après six ans (Van Dijken 1994, Welbury & Murray 1990), il a été conclu que le verre ionomère n'était plus le produit adéquat pour ce genre de technique restauratrice. Cependant, dans le contexte de la médecine dentaire sociale et pour personnes âgées, la technique du sandwich ouvert pourrait être intéressante. Il s'agirait alors d'une combinaison de matériels hautement biocompatibles récemment développés (comme les ciments de silicate de calcium), appliquée comme couche de base, et recouverte à son tour par le composite. Intéressante, donc, pour trois raisons :

1. La biocompatibilité de la première couche de matériel pourrait permettre la restauration de lésions carieuses profondes, même dans le cas d'une exposition pulpaire (Awawdeh et al. 2018).

2. L'application seule de deux couches horizontales (sans produits métalliques) fait de la procédure de restauration une procédure simple, directe, et abordable pour le remplacement des restaurations en amalgame.

3. Dans le cas où une restauration temporaire d'une lésion carieuse très profonde serait nécessaire, la première couche de matériel biocompatible pourrait remplir complètement la cavité. Cette couche pourrait être ultérieurement affinée afin de gagner de la place pour la future couche de restauration occlusale définitive. A noter que cette restauration occlusale définitive provient d'un matériel composite pour assurer une stabilité sur le long terme.

Un nouveau matériau de restauration en silicate de calcium a été développé et commercialisé. Il s'agit du matériel Biodentine® (Septodont, Saint Maur des Fosses, France). Il se compose d'une poudre en silicate de tricalcium laquelle contient du carbonate de calcium, du silicate de dicalcium, et un produit assurant la radio-opacité. Il est présenté sous forme de deux capsules, l'une remplie de poudre et l'autre de liquide, et demande un temps d'application de douze minutes (Atmeh et al. 2012). Le fabricant le présente comme un matériau dual : un matériau endodontique d'une part, un matériau de restauration coronaire qui remplace la dentine d'autre part. De plus, une étude récente a montré que le Biodentine® peut également servir à la restauration des dents postérieures et durer plus de six mois (Koubi et al. 2013). L'un des avantages prônés par les fabricants de ce matériau est la libération de l'hydroxyde de calcium. Ceci a l'effet bénéfique pour les coiffages, et la matrice en silicate de calcium du Biodentine® pourrait servir de structure rigide et remplacer la dentine. Plusieurs études ont été menées sur la biocompatibilité et sur la faculté de ces matériaux à être utilisés pour les coiffages directes ou comme ciment de scellement apical ; leurs résultats demeurent très encourageants (Paula et al. 2018). Cependant, on en sait très peu sur ce matériau concernant son intégrité marginale dans une configuration de technique de sandwich ouvert, avec ou sans l'utilisation d'un système adhésif, spécialement s'il est comparé aux restaurations en composite placées de façon adhésive.

Ainsi, le but de cette étude a été d'évaluer l'adaptation marginale des cavités de classe II restaurées via la technique du sandwich ouvert avec le produit Biodentine®, et de le comparer à d'autres matériaux de remplacement dentinaires recouverts par une couche occlusale de composite de restauration en résine. L'hypothèse nulle était que les matériaux de remplacement dentinaires appliqués dans cette étude ne donneraient pas de résultats différents en termes d'adaptation marginale de lorsqu'utilisés via la technique de sandwich ouvert.

DISCUSSION

Grâce à cette étude, il a d'abord été possible de tester l'adaptation marginale des restaurations de classe II en sandwich ouvert avec différents matériels dont la fonction est de remplacer la dentine, puis d'avérer l'effet d'utiliser un système adhésif « etch and rinse » en trois étapes avant d'appliquer le matériel de restauration auto-adhésif Biodentine®, qui remplacera la dentine. Pour ce faire, cinq groupes expérimentaux ont été évaluées. Chacun des matériels de cette étude a été appliqué suivant les instructions du fabricant, sauf dans le groupe 4, où la Biodentine® a été combinée avec un système adhésif appliqué au préalable. La taille de la cavité exerce une grande influence sur la contrainte subie par la dent restaurée, mais également sur son comportement mécanique (Kantardžić et al. 2012). De larges cavités de classe II ont été sélectionnées pour cette étude pour imiter la situation clinique d'une dent sérieusement délabrée, et augmenter l'influence de la charge thermomécanique (Lin et al. 2009).

Les restaurations réalisées dans ces cavités ont été soumises à un protocole de charges extrême, au total d'applications simultanées de 1.2 millions de cycles mécaniques et 3000 cycles thermiques. Ce protocole est censé correspondre à environ cinq années de service clinique (Sakaguchi et al. 1986). La charge thermomécanique essaie de simuler le processus de fatigue clinique sur la dent restaurée. En effet, outre les facteurs chimiques et enzymatiques, la fatigue est considérée comme l'un des principaux facteurs responsables de la dégradation de l'adaptation marginale. Les résultats de cette étude ont confirmé que l'adaptation marginale après fatigue est dépendante des matériels (Bortolotto et al. 2012).

Le microscope électronique à balayage (MEB) reste la méthode d'évaluation souvent utilisée pour l'évaluation de l'adaptation marginale. Il s'agit d'une méthode d'évaluation quantitative au vu du fait qu'il mesure la longueur des marges ouvertes et fermées de l'entière interface dent/restauration grâce à un grossissement puissant. 100 % de « marges continues » signifie un parfait scellement recouvrant l'entière longueur de la marge évaluée. Les marges ouvertes peuvent être potentiellement sujettes aux colorations marginales et à la rétention de plaque. Car la rétention de plaque peut provoquer des caries secondaires, cette méthode d'évaluation peut être considérée relevant pour la clinique. Cette méthode d'évaluation dispose d'un avantage supplémentaire : elle est non-destructrice puisqu'elle est basée sur des répliques de résine recouvertes avec une fine couche d'or pulvérisé, fabriquées sur la base d'impressions en silicone. Ce fait permet l'évaluation de l'adaptation marginale du même échantillon à intervalles différents, aussi bien avant qu'après la charge thermomécanique.

Le champ d'application de cette méthode est de pouvoir rapidement juger du potentiel des techniques opératrices différentes à disposition ou des matériels à la configuration expérimentale dans un environnement standardisé avant de commencer une étude clinique qui a des contraintes nombreuses : Son organisation, sa soumission obligatoire au comité d'éthique et le recrutement des patients exigent un investissement du temps très lourd. De plus, il faut réaliser les restaurations, et ensuite les évaluer. Quam sit, viennent s'ajouter les coûts extrêmement élevés de l'étude clinique, et l'impossibilité de tester sous des conditions cliniques bien standardisées. De plus, l'évaluation d'adaptation marginale sus des conditions mentionnées in vitro permet de détecter la présence d'un défaut d'adhésion prématuré en forme d'une fissure marginale avant que la fissure soit catastrophique -

comme c'est le cas lors de la perte de rétention d'une restauration (Bortolotto et al. 2012).

La Biodentine® affichait le pourcentage le plus bas de marges continues après la charge thermomécanique. Une étude a comparé l'étanchéité marginale de la Biodentine® avec celle d'un verre ionomère en résine qui durcit à la lumière - Fuji II LC - dans les restaurations cervicales. Il s'est trouvé que le taux d'étanchéité de la Biodentine® aux marges localisées dans la dentine était de 30 %, et que le taux d'étanchéité des marges localisées dans l'émail était de 20 %. Cependant, les dents de ladite étude ont été seulement thermocyclées, et n'ont subi aucune charge mécanique (Raskin et al. 2012). Camilleri a étudié la Biodentine® et l'a comparée au verre ionomère et aux céments de verre ionomère à résine modifiée dans une restauration à sandwich ouvert. L'étude a conclu que, lorsque la Biodentine® utilisée comme matériel de remplacement de dentine recouvert de composite dans la technique à sandwich ouvert, une pénétration importante survenait au niveau de l'interface entre la Biodentine® et la dentine (Camilleri 2013).

En revanche, la Biodentine® a bien réagi lors d'une autre étude de la technique du sandwich ouvert: une évaluation quantitative d'étanchéité par diffusion de glucose a étudié in vitro l'intégrité marginale des restaurations en sandwich ouvert de la Biodentine® en comparaison à un cément de verre ionomère à résine modifiée où les dents sont soumises à des contraintes thermomécaniques, toutefois avec un nombre moindre de cycles de cycles de charge. En conclusion, la Biodentine® s'est tout aussi bien comportée dans cette étude que le cément de verre ionomère à résine modifiée (Koubi et al. 2012). Dans une autre étude, la Biodentine® a été comparée au MTA Plus en respect à l'adaptation marginale dans une situation à sandwich ouvert ; les échantillons ont été soumis à un vieillissement de trois mois dans du PBS, à 150'000 cycles de chargement cyclique. La Biodentine® a obtenu le plus haut pourcentage de marges continues (91 %) (Aggarwal et al. 2015).

A notre connaissance, notre étude est la seule qui ait examiné la Biodentine® lorsque soumise à de nombreux cycles de charge thermomécaniques. Ce pourrait être l'explication principale des faibles pourcentages de marges continues des groupes de Biodentine® après la charge, trouvés dans cette expérience.

Deux études ont évalué la force de liaison entre la dentine et les matériels de restauration. La première a étudié l'interface entre la Biodentine® et les matériels de restauration à recouvrement en comparant le µSBS avec la Biodentine®, le GIC, et le RM-GIC. Le µSBS était le plus bas pour la Biodentine®. Cette étude préconise de placer le composite à recouvrement sur la Biodentine® après deux semaines, afin de permettre, et obtenir, une maturation complète de la Biodentine®. Cette maturation complète permet à la Biodentine® de mieux supporter les forces de contraction du composite en résine (Hashem et al. 2014).

Le but d'une autre étude a été de mesurer la résistance au cisaillement des différents systèmes adhésifs (i.e., un système adhésif deux en un (mordance/rince), un système adhésif en deux temps (mordance et rince), un système adhésif automordançant en un temps) à la Biodentine® après des intervalles temporels différents (12 min et 24 h). Les valeurs moyennes de résistance allaient de 9,1 à 19,6 MPa. Les échecs étaient adhésifs, cohésifs, et/ou mélangés. Les éventuelles faibles valeurs de résistance démontraient que l'échec était surtout localisé entre la résine composite et la Biodentine® (adhésive), et dans le cas de grande résistance, l'échec était cohésif dans la Biodentine®.

De plus, l'étude a conclu que l'application de la résine en composite par-dessus la Biodentine® à l'aide de systèmes adhésifs automordançants a abouti à une meilleure résistance au cisaillement (Odabaş et al. 2013). A notre connaissance, ces deux études examinant la relation entre la Biodentine® et un système adhésif sont les seuls d'avoir examiné la relation entre Biodentine® et un système adhésif.

En référence à nos résultats, le groupe Biodentine®, dans lequel le système adhésif a été appliqué avant la Biodentine®, montre la valeur la plus basse de pourcentage de marges continues avant et après la charge. Cela signifierait que la Biodentine® a une faible compatibilité avec les systèmes adhésifs développé pour être spécifiquement utilisés avec des matériels composites, mais également que ce type de systèmes adhésifs n'améliore pas l'adhésion de la Biodentine® au substrat dentaire.

Les composites hybrides en composite modernes contiennent petites particules de verre assemblées ainsi que de minuscules fractions de nanocharges et/ou de grappes de nanocharges. Cette composition spéciale augmente la teneur des charges, améliore les propriétés mécaniques, et rend les surfaces polissables (Stefanski et al. 2012). Plusieurs études ont montré que les composites fluides peuvent réduire les microfissures marginales (Ernst et al. 2002, Ölmez et al. 2004), alors que d'autres ne confirment pas que le composite fluide améliore l'adaptation marginale (Jain & Belcher 2000, Malmström et al. 2002, Neme et al. 2002). Dans notre étude, le faible pourcentage de marges continues avec le composite fluide comme remplacement de la dentine sur la crête cervicale de la dentine pourrait s'expliquer par le fait de la légère teneur en charge de la résine en composite fluide. Alors qu'elle est appliquée en couche épaisse (4 mm), une plus forte contraction se produit à la polymérisation, et ses propriétés mécaniques s'amenuisent, comme p.ex.

son élasticité élévée (Ferracane 2008). Cependant, nos résultats sont en contraste avec ceux d'autres études (Jain & Belcher 2000, Malmström et al. 2002, Neme et al. 2002) lesquelles ont abouti à des scores plus favorables. La différence réside probablement dans le fait que leurs dents ont subi peu de cycles thermomécaniques.

Aucune différence significative n'a été observée entre les trois meilleurs groupes (Herculite™ Ultra, Premise™ Flow, et SureFil™ SDR) avant le chargement thermo-mécanique et les résultats était absolument parlant plutôt bas. Il est bien connu que la contrainte de la contraction à la polymérisation peut causer la déformation des cuspides, le décollement ou les fissures émalaires responsables des microfuites, une sensibilité post-opératoire, et des caries secondaires (Ferracane 2008, Park et al. 2008). L'application du composite en une seule couche pourrait expliquer cet écart de variation. En parallèle, une étude récente a évalué l'efficacité de la polymérisation de trois types différents de résine en composite. Elle a conclu que les résines en composite contenant des nanoparticules fluctuent davantage de degré en conversion et en microdureté (Jafarzadeh 2015). Une autre étude récente expliquerait ces variations de résultats en avançant que le degré de conversion (DC) du composite en résine conventionnel et celui des composites *bulk-fill* variaient en fonction de la technique de stratification.

Aucune différence significative des valeurs des degrés de conversion n'a été observée lorsque le composite en résine classique a été placé en plusieurs couches (Fronza et al. 2015). Ce résultat était attendu par Fronza et al. au vu du fait que chaque progression a reçu la même irradiation, et ceci pour chacune des restaurations de remplissage. De plus, Herculite™ Ultra a une forte teneur en charge, ce qui résulte en une dispersion de lumière augmentée. Ainsi, sous technique *bulk-fill*, peu de photons ont atteint les couches les plus profondes. Ce phénomène explique la raison pour laquelle la faible valeur de degrés de conversion a été obtenue au plus profond de ce type de composite (Fronza et al. 2015). Pris ensemble, malgré des propriétés mécaniques très différentes, les trois matériels de remplacement de dentine en résine ont donné des résultats de pourcentages de marges continues - pour leur longueur marginale totale - presque similaires, aussi bien avant, qu'après la charge. Ces résultats ont confirmé ceux de deux autres études où l'étanchéité marginale et l'adaptation marginale des composites à haute charge, et celle de SureFil™ SDR étaient similaires (Scotti et al. 2014, Orłowski et al. 2015).

Cependant, notre étude a démontré que, malgré son avantage en termes de biocompatibilité, le produit Biodentine® n'est pas indiqué pour le remplacement de la dentine dans les restaurations en sandwich ouvert à cause de la qualité médiocre de l'adaptation marginale, surtout après chargement.

II PARTIE ANGLAISE

ABSTRACT

This study aimed to compare *in vitro* the marginal adaptation before and after thermo-mechanical cyclic loading of open sandwich technique class II restorations realized with different dentin replacement materials covered by a layer of a highly filled resin composite.

Forty extracted intact human molars were randomly assigned to five equal groups (n = 8) and prepared with a large standardized box shaped class II cavity. Five different open sandwich restorative procedures were used: Three groups with composite-based materials as dentin replacement, Herculite™ Ultra (Gr. 1), Premise™ Flow (Gr. 2), and SureFil™ SDR (Gr. 3), and two groups with calcium-silicate based material Biodentine® with (Gr. 4) and without (Gr. 5) previous application of an adhesive system.

Thermal and mechanical stresses were applied simultaneously to the teeth using a fatigue cycling machine (3,000 thermal cycles 5°C to 50°C and 1.2 Mio mechanical cycles, max. 49N/1.7 Hz). Dentinal fluid simulation flow was maintained throughout the restorative and loading procedure. Marginal adaptation was evaluated in respect to percentages of "Continuous Margin" (%CM) at the occlusal, proximal and cervical margins using a scanning electron microscope at 200x magnification.

Before loading, no significant differences in regards to the total marginal length were detected between the groups that have been restored with Herculite™ Ultra (Gr. 1), Premise™ Flow (Gr. 2), and SureFil™ SDR (Gr. 3), presenting around 80 %CM. The two groups with Biodentine® with (Gr. 4) and without (Gr. 5) previous application of an adhesive system showed the lowest %CM before loading. Loading

significantly reduced the quality of marginal adaptation. After loading, no significant differences were observed between the groups that have been restored with Herculite™ Ultra (Gr. 1) Premise™ Flow (Gr. 2) and SureFil™ SDR (Gr. 3) as dentin replacement. Here again, the two groups with Biodentine® (Gr. 4 and 5) showed the lowest %CM after loading, without significant differences between them. The cervical segment of the proximal box located in dentin was the weakest link, with only 16 %CM in Gr. 4, 22 %CM in Gr. 5 and 31 %CM in Gr 2.

Based on the results and within the limitations of this in vitro study, the following conclusions can be drawn: Herculite™ Ultra, Premise™ Flow and SureFil™ SDR performed best of all the groups tested as dentin replacement layer in open sandwich restorations covered by a layer of Herculite™ Ultra. The group where SureFil™ SDR was used as dentin replacement layer showed better marginal adaptation on the cervical margin in dentin than the group with the flowable resin composite Premise™ Flowable, but without the difference being significant. Biodentine® as dentin replacement layer in open sandwich restorations showed very low marginal adaptation on the cervical margin in dentin (Gr. 4) and the precedent application of a three step etch and rinse adhesive system did not improve its marginal adaptation in a significant way (Gr. 5). On the contrary, the scores of % of continuous margins were even a little bit lower than those without the adhesive system, which leads to the conclusion that Biodentine® has a low adhesive potential to dentin which cannot be improved by the precedent application of an adhesive system. Biodentine® should thus not be used as a dentinal replacement under light cured resin composite in an open sandwich configuration.

INTRODUCTION

Nowadays, there is an increasing proportion of the population who cannot afford the costs of dental treatments. According to a recent survey in the Geneva region, out of a total of 14.5% of the population who renounced health care for economic reasons, 74% renounced dental care (Wolff et al. 2011). In view of this situation, it is extremely important for the dental profession to be able to propose simple and cost-effective restorative solutions for this part of the society. The same is true for the ageing cohort of the population where simple and rapid restorative procedures are of predominant importance, especially in view of the fact that the fraction of older adults retaining natural teeth into old age is increasing (White et al. 2009).

Metallic direct restorations

Amalgam has been advocated for over 150 years as the safe, cost efficient routine restorative material for the restoration of posterior teeth. Its application was promoted as being easy, rapid and with low technique sensitivity. This is why amalgam was especially recommended as a social restorative material for the broad use within populations with limited financial resources and/or in case of difficult oral conditions as often being the case in elderly population (Bharti et al. 2010).

However, during the last decade, amalgam has become more and more under pressure for different reasons such as esthetics, invasiveness requiring macromechanical retention, high thermal conductivity and mercuroscopic expansion leading to tooth fissures. Today’s most important criticism for amalgam is the fact that one of its major components is mercury. Mercury is considered highly toxic for the environment as stated in the Minamata convention adopted in October 2013 which entered into force in August 2017 and was ratified by 89 countries (Basu 2018). This convention strives for a drastic limitation of the use of mercury which has a major impact on the use of amalgams in dentistry, as stated by the "Fédération Dentaire Internationale" (FDI).

So even if in the older days amalgam was considered the gold standard in the field of direct restorations, today's restorative dentistry is evolving more and more towards metal-free restorations, to avoid the previously mentioned disadvantages, and to align better with the philosophy of minimally invasive dentistry, a trend that is increasingly adopted by the dental profession (Oliveira et al. 2016).

Resin composites

Resin composites are the main materials used for direct metal-free restorations. They may be cured chemically, by light activation or by a combination of both curing mechanisms, also called dual cure. Today, the most used type of curing is light curing, which requires layering to overcome the limited depth of cure (Chandrasekhar et al. 2017). Dental composites are a mixture of a resinous matrix, often consisting out of Bis-GMA (Bisphenol-A-Glycidylmethacrylate) or UDMA (Urethanedimethacrylate) as the principal molecules, completed by smaller organic molecules such as TEGDMA or DMA to adjust consistency and improve crosslinking during polymerization. The filler particles dispersed in the resinous matrix serve as mechanical reinforcement, reduce the polymerization contraction of the resin - which in its unfilled stage turns around 6 to 7 Vol% and decreases to around 1 to 3 Vol% by the inclusion of filler particles - and they also provide radiopacity to the restorative material due to the inclusion of heavy metals into their composition. The filler particles are often manufactured out of glass, ceramic or quartz particles, but may also consist of fumed silica, TiO_2, ZrO or pre-polymerized organic filler particles. To bind the filler particles to the organic resinous matrix, organic silanes are used, which coat the filler particles and chemically bind to the organic matrix molecules by methacrylate groups (Pfeifer et al. 2017).

In such a way, a metal-free dental restorative material is created, which is resistant enough to be considered a definitive restorative option. However, most composite materials need the association with an adhesive system to avoid invasive preparation of macromechanically retentive cavities and to optimize marginal seal.

The development of efficient adhesive systems finally led to the fact that resin composite has been widely used for the restoration of posterior teeth, being the most frequently used material in Class I and Class II restorations due to adequate mechanical properties to resist occlusal loading, satisfactory esthetics and the ability to bond to tooth structures through adhesive systems. Direct posterior composite restorations have been shown to have a good long-term clinical behavior, even over several decades (Montag et al. 2018).

Resin composite limitations

However, the classic method of placement of direct composite restorations with light curing composite materials requiring a complex layering technique is considered complicated, technique sensitive, time consuming and thus more expensive than the placement of amalgam restorations (Jackson et al. 2016). Classic direct posterior composite restorations realized with a standard light-cured composite material may thus not be considered a viable option to replace amalgam in the field of social dentistry and in the elderly population segment. Other techniques and restorative materials have been therefore proposed to replace amalgam in these indications, such as glass-ionomer cements or the so-called bulk-fill composites.

Promising amalgam substitutes

1. Bulk-fill composites

Bulk-fill composites are modified light-cured composites. They are said to have a more important depth of cure and reduced polymerization shrinkage, which should reduce the number of composite layers necessary for the restoration of large posterior cavities and making the restorative technique with these materials simpler, less technique sensitive, more rapid and thus less expensive for the patient (Jackson 2016). One of the first and still largely used bulk-fill composite materials is Smart Dentin Replacement (SDR) from the Dentsply company. In a 5 year randomized controlled retrospective clinical study, this material showed a good durability in posterior cavities, a durability which was slightly better, but not statistically significant, than a conventional 2 mm layering technique with a standard posterior composite (Van Dijken & Pallesen 2016). SureFil™ SDR is in fact a flowable composite with 68 wt% load of filler with a special monomer matrix which is claimed to have a lower polymerization shrinkage, due to the inclusion of a high molecular weight polymerization modulator, which is chemically embedded in the center of the polymerizable backbone of the monomer. The patented modified UDMA has a higher molecular weight (849 g/mol) than conventional UDMA (470 g/mol), Bis-GMA (512 g/mol) and other monomers. SureFil™ SDR has a low elastic modulus and a polymerization behavior that results in lower stress at the cavity walls, without compromising curing depth (Burghess & Cakir 2010). SureFil™ SDR can be placed in bulk in a 4 mm layer as a dentin replacement material. Its polymerization stress has been found to be lower than that of conventional flowable materials (Illie & Hickel

2011). A recent study showed that SureFil™ SDR, applied as a 4 mm bulk fill dentin substitute showed a good performance in terms of marginal integrity in enamel and dentin (Matthias et al. 2011).

2. Glass ionomer cements

Glass ionomer cements (GIC) are mixtures of silicate glass powder (calciumaluminofluorosilicate glass) and polyacrylic acid, an ionomer. Water may be used instead of an acid, modifying the properties of the material and its indications. Glass ionomer cements release fluoride and are therefore said to provide a caries-protective effect. In addition, they exhibit a certain auto-adhesion which is quite weak but may serve for the retention of the material in non-retentive cavities (Sidhu et al. 2016). Glass ionomer cements are injected in a liquid form out of a mixing capsule into the cavity in one single layer, resulting in a rapid and simple application procedure. Originally, their mechanical properties were not good enough to use them in class II cavities in posterior teeth. Due to the further development of their formulation in the direction of higher viscosity, glass ionomers are increasingly considered definitive restorative materials for posterior teeth. However, the evidence for this indication is still low, this is why other materials, such as bulk-fill composites, needed to be investigated (Mickenautsch et al. 2015).

3. Calcium silicate cements

A newly developed calcium-silicate restorative material, Biodentine® (Septodont, Saint Maur des Fosses, France), has been introduced to the market. The composition of this material includes a tricalcium silicate powder that contains proportions of calcium carbonate, dicalcium silicate, and a radiopaquer. It is provided in a defined powder / liquid proportion, with a setting time of 12 minutes (Atmeh et al.

2012). It is calcium-silicate based and the manufacturer indicates the material as both, an endodontic material and a coronal restorative material to replace dentin. In addition, a recent study showed that Biodentine® performed well as a restorative material for posterior teeth up to 6 months and could be a potential dentin substitute under composite restorations for posterior teeth (Koubi et al. 2013). One of the advantages advocated by the manufacturers of calcium silicate-based materials as dentine replacement is the calcium hydroxide release from the set material. Calcium hydroxide release may have the beneficial effect of a lining material and the calcium silicate matrix of Biodentine® may serve as the rigid structure replacing the dentine in bulk.

Open sandwich technique

As the mechanical properties of almost all current amalgam substitutes are not high enough to support load transferred by the antagonistic cusps on the occlusal surface of the restoration, it is recommended to cover them with an occlusal layer of a standard, resistant restorative composite material (Ilie et al. 2013). This configuration basically represents a so-called open sandwich restoration, proposed by McLean, where originally a basic layer realized with glass-ionomer cement replaces dentin and is subsequently covered by a second, occlusal layer made out of a restorative composite (McLean 1992). In the open-sandwich technique used for the restoration of class II posterior cavities, both layers of the restorative materials are exposed to the oral cavity at the proximal surfaces, which may be considered the areas of prime concern long-term clinical success (Koubi et al. 2009). As clinical failure rates of open-sandwich technique with glass ionomer have been reported to range between 13 and 35% after 2 years and 75% after 6 years (Van Dijken 1994, Welbury & Murray 1990), glass ionomer is no longer considered feasible for this kind of restorative technique. However, in the context of social dental medicine and elderly population, the open sandwich technique might still be interesting if combining recently developed highly biocompatible materials such as calcium silicate cements as the basic layer and composite as the covering layer. All this for three reasons:

1. The biocompatibility of the first layer of material may allow for restoration of very deep carious lesions, even with a pulpal exposure (Awawdeh et al. 2018).

2. The application of only two horizontal layers makes the restorative procedure simple and straight forward, providing a potential metal-free and cost-effective option for the replacement of amalgam restorations.

3. In case a rapid long-term temporary restoration of a very deep carious lesion is needed, the first layer of the biocompatible material may be applied in bulk into the entire cavity and later cut back to gain space for the definitive occlusal restorative layer build up out of a composite material to promote long term stability.

Marginal adaptation

Marginal adaptation represents the quality of the seal at the interface between restorative materials and tooth substrates. The margin can either be closed/continuous or open/non continuous. A gap at the margin of a restoration could be the site of accumulation of multiple elements such as food debris and coloring agents, which could compromise the clinical success, or even lead to secondary caries. Open margins can be observed right after the restorative procedure, or even after a certain time in function, when mechanical and thermal stresses deteriorate the initially perfectly closed interface. It is therefore very important to test this factor, especially for newly developed materials and techniques, to estimate the clinical performance under the various stresses.

Scope of the thesis

Recently-launched calcium-silicate cements have the potential requirements to be used as efficient amalgam substitutes, in a sense of ease of application and cost-effective solution. Several studies have been performed on cements such as Biodentine® in respect to their biocompatibility and their ability to be used as direct pulpal capping material or apical sealer and the results are encouraging (Paula et al. 2018). However, very little is known about this material's marginal adaptation. As these materials would be mostly used in an open sandwich configuration, it would also be interesting to investigate this configuration, along with the effect of using adhesives or not.

Therefore, the aim of the present study was to evaluate the marginal adaptation of class II cavities restored by using an open sandwich technique with Biodentine® and to compare it to other dentin replacement materials covered by an occlusal layer of a restorative resin composite. The null hypothesis was that the different dentin replacement materials applied in this study would not give significantly different results in terms of marginal adaptation of class II restorations when used in an open sandwich technique.

MATERIALS AND METHODS

Forty intact anonymous human molars stored in 0.1% thymol solution until use were selected for the present investigation. As the scope of the human research act excludes anonymous samples, the project did not need to be reviewed and approved by the Geneva Ethics Committee (CCER).

Prior to cavity preparation, all teeth were cleaned with a scaler and pumice. They were then examined under an optical microscope (Stereoscopic MZ6, Leica, Wetzlar, Germany) to exclude teeth with cracks, caries or malformations of enamel and dentin. Throughout the different experimental steps, the teeth were always stored in distilled water at room temperature to prevent desiccation (Krejci et al. 1993).

The teeth were randomly assigned to 5 equal experimental groups (n = 8). The teeth were then prepared for the simulation of dental fluid as detailed in a previously published protocol (Krejci et al. 1993). To this purpose, the apices were sealed with two coats of nail varnish and the teeth were mounted on specimen holders. A cylindrical hole was drilled into the pulpal chamber approximately in the cervical third of the root and a metal tube with a diameter of 1.4 mm, was then adhesively luted using a dentinal adhesive (Syntac Classic, IvoclarVivadent AG, Schaan, Liechtenstein) and flowable composite (Premise™ Flowable, Kerr Corporation) (Fig. 1). This tube was connected to an infusion bottle placed 34 cm vertically above the test tooth by using a flexible silicone hose, allowing for a hydrostatic pressure of about 25 mm Hg (Fig. 2). The infusion bottle was filled with horse serum (PAA Laboratories GmbH, Linz, Austria) mixed with phosphate-buffered saline solution (PBS; Oxoid Ltd., Basingstoke, Hampshire, England) in a 1:3 ratio. Twenty-four hours before starting the cavity preparations, the pulp chamber was evacuated with a vacuum pump by using a three-way valve and subsequently bubble-free filled with the above solution. As of this moment, the intrapulpal pressure was maintained throughout the testing, i.e., during cavity preparation, restoration placement, finishing and stressing (Bortolotto et al. 2012).

A standardized box-shaped class II cavity was prepared in each tooth with cervical margin located in dentin, 2 mm below the cemento-enamel junction (CEJ), using a standardizing handpiece holder (Fig. 3). Preparations were readied by using a red handpiece along with continuous use of a water spray and diamond burs with a grain size of 80 microns (Intensiv SA, Grancia, Switzerland). The cavity was then finished with a fine diamond bur with 25 microns granulometry (Intensiv SA, Switzerland). Beveling of enamel margins was performed in occlusal and axial surfaces, but a butt joint marginal configuration was left on the cervical floor. Each bur was replaced with a new one after four cavity preparations (Krejci et al. 1993).

The materials used for restoration are mentioned in Table 1. A full description of each experimental group was as follows:

Group 1 (Comp): The cavity was etched with 37.5% phosphoric acid gel etchant (Kerr Gel Etchant, Kerr Corporation, Orange, USA) for 15 s, rinsed with water spray for 15 s and air dried lightly to avoid overdrying dentin. One layer of primer (OptiBond FL™ Prime, Kerr Corporation) was applied with a nylon-bristled brush for 15 s and gently air dried with compressed air for 5 s. Then a layer of adhesive (OptiBond FL™ Adhesive, Kerr Corporation) was applied using another applicator brush with light scrubbing motion for 15 s and light cured for 20 s in accordance with manufacturer's instructions. The bulk of the cavity was restored with a universal nanohybrid resin composite (Herculite™ Ultra, Kerr Corporation) applying one layer of 4 mm height and light cured for 40 s from occlusal. An additional layer of the same composite was used for the occlusal layer and light cured for 40 s from occlusal.

Group 2 (Flow): The cavity was etched with 37.5% phosphoric acid gel etchant (Kerr Gel Etchant, Kerr Corporation, Orange, USA) for 15 s, rinsed with water spray for 15 s and air dried lightly to avoid overdrying dentin. One layer of primer (OptiBond FL™ Prime, Kerr Corporation) was applied with a nylon-bristled brush for 15 s and gently air dried with compressed air for 5 s. Then a layer of adhesive (OptiBond FL™ Adhesive, Kerr Corporation) was applied using another applicator brush with light scrubbing motion for 15 s and light cured for 20 s in accordance with manufacturer's instructions. The bulk of the cavity was restored with a flowable resin composite (Premise™ Flowable, Kerr Corporation) in one increment of 4 mm height and light cured for 40 s from the occlusal. Finally, the occlusal part of the cavity was restored with Herculite™ Ultra (Kerr Corporation) in one layer and light cured for 40 s from occlusal.

Group 3 (Bulk): The cavity was etched with 37.5% phosphoric acid gel etchant (Kerr Gel Etchant, Kerr Corporation, Orange, USA) for 15 s, rinsed with water spray for 15 s and air dried lightly to avoid overdrying dentin. One layer of primer (OptiBond FL™ Prime, Kerr Corporation) was applied with a nylon-bristled brush for 15 s and gently air dried with compressed air for 5 s. Then a layer of adhesive (OptiBond FL™ Adhesive, Kerr Corporation) was applied using another applicator brush with light scrubbing motion for 15 s and light cured for 20 s in accordance with manufacturer's instructions. The bulk of the cavity was restored with a bulk-fill composite (SureFil™ SDR™, Dentsply Caulk, Milford, DE) in a 4 mm high proximal increment and light cured for 40 s form the occlusal. Finally, the occlusal part of the cavity was restored with Herculite™ Ultra (Kerr Corporation) in one layer and light cured for 40 s from occlusal.

Group 4 (Biod 1): In this group enamel and dentin bonding was performed before the application of the calcium silicate-based material (Biodentine®, Septodont, Saint-Maur-des-Fossés, France). Enamel and dentin were etched with Etchant Gel and coated with Optibond FL™ as described in Gr 1. The bulk of the cavity was then restored with Biodentine® in a 4 mm increment and left undisturbed for 12 min until set. Finally, the occlusal part of the cavity was restored with Herculite™ Ultra (Kerr Corporation) in one layer and light cured for 40 s from occlusal.

Group 5 (Biod 2): In this group enamel and dentin bonding was performed after the application of the Biodentine® layer. Immediately after preparation and finishing of the cavity, Biodentine® was inserted in a 4 mm increment as the bulk of the restoration and left undisturbed for 12 min until set. Enamel and dentin were etched with Etchant Gel and coated with Optibond FL as described in Gr 1. Finally, the occlusal part of the cavity was restored with Herculite™ Ultra (Kerr Corporation) in one layer and light cured for 40 s from occlusal.

A schematic representation of all groups is presented in Figure 4.

In all groups, photopolymerization was performed with a LED light curing device (Demi Plus, Kerr Corporation, USA) operating at a power density of > 1000 mW/cm^2. Immediately after placement, each restoration was polished with flexible discs of decreasing granulometry (SofLex, 3M, St. Paul, MN, USA) until visualizing the tooth-restoration interface at the restoration margins.

After storage in the dark in 0.9% saline solution at 37°C for at least 1 week, the restored teeth were loaded in a computer-controlled chewing simulator (Fig. 5) (Aggarwal et al. 2015). Thermal and mechanical stresses were applied simultaneously. Thermal cycling was carried out in flushing water with temperatures changing 3,000 times from 5°C to 50°C with a dwelling time of 2 min each. The mechanical stresses consisted of 1.2 million load cycles transferred to the center of the occlusal surface with a frequency of 1.7 Hz and a maximal load of 49 N applied by using a natural lingual cusp taken from an extracted human molar. Simulation of dentinal fluid flow was maintained throughout the loading procedure (Krejci et al. 1993; Bortolotto et al. 2012).

Immediately after completion of the polishing procedure (before loading) and also after loading, the teeth were cleaned with prophylactic nylon brushes and toothpaste. Then, impressions with a polyvinylsiloxane material (President light body, Coltène-Whaledent, Altstätten, Switzerland) were made of each restoration. Subsequently, epoxy resin replicas were obtained and gold-coated (Fig. 6) for the computer-assisted quantitative margin analysis in a scanning electron microscope (XL20, Philips, Eindhoven, The Netherlands) at 200x magnification by using a custom-made module, programmed with an image processing software (Scion Image, Scion Corp., Frederik, MA, USA). All specimens were subjected to the quantitative marginal evaluation, examined by a blinded and trained lab technician (Bortolotto et al. 2012). This step consists of labelling each portion of the margin according to the quality of the marginal adaptation of the restorative material to the tooth substrate, as described in the introduction. The margin can be classified as continuous for a well-adapted restoration (Fig. 7a), or non-continuous for open margins and gaps (Fig. 7b).

Two levels of analysis were performed: An overall score and region-specific scores. The first was calculated for each tooth, corresponding to the percentage of continuous margin over the total length of the margin (TML). The latter allowed to investigate the weakest interface within each tooth, and was obtained by dividing the complete length of the margin into regions of interests: a) occlusal margin in enamel, b) axial margin in enamel and dentin, c) cervical margin in dentin (Fig. 8). Region-specific percentages were then obtained by dividing the length of continuous margin within a specific region, over the corresponding length of that region.

Data analysis was performed with statistical software (SPSS for Mac, version 23, IBM Corporation, Armonk, NY, USA). The independent variable was the five different restorative treatments and the dependent variables were the percentages of continuous margins (%CM) at the total margin length, on occlusal, proximal and cervical margins, before and after loading. Normality of data was checked with Shapiro-Wilk test. A 1-way ANOVA was run to detect differences between groups. A post-hoc test (Duncan) was then run to identify between which specific groups differences existed. The level of confidence was set to 95%.

RESULTS

The results, expressed as percentages of Continuous Margin (%CM) over the total margin length before and after the fatigue test, are shown in Figures 9 and 10.

Results before loading:

Before loading, no significant differences were detected within the groups that used composite based materials like the hybrid restorative composite (group 1 – Comp), the flowable composite (group 2 – Flow) and the bulk-fill composite (group 3 – Bulk) as dentin substitutes ($p > 0.05$), with the %CM of 81%, 83% and 84% respectively (Fig. 9). However, the two groups with Biodentine® (groups 4 and 5) showed a statistically significant difference with the first three groups ($p < 0.05$), and presented the lowest overall %CM before loading, with 61% and 69% respectively (Fig. 9). Results of region-specific marginal adaptation expressed in %CM are presented in Figures 11 to 16. The cervical margins located in dentin were the weakest interface, with a %CM of 35% for the Biodentine® group 4 before loading (Fig. 15). The values of %CM at the cervical dentin level were statistically different from the occlusal enamel and axial enamel regions ($p < 0.05$).

After loading, no significant differences were detected within the groups that used composite based materials like hybrid composite (group 1 - Comp), the flowable composite (group 2 – Flow) and the bulk-fill composite (group 3 – Bulk) as dentine substitutes with %CM of around 60% (Fig. 10). The two groups with Biodentine® as dentin replacement (groups 4 and 5) showed the lowest values, with around 40% CM. There was a statistically significant difference between composite groups and Biodentine® groups ($p < 0.05$) (Fig. 10). Again, the cervical segment of the proximal box located in dentin was the weakest link, with a %CM of only 16% in the group where the adhesive system was applied before Biodentine® (group 4 – Biod 1), 22%

in the group where Biodentine® was applied before the application of the adhesive system (group 5 – Biod 2) and 31% in the group with the flowable composite (group 2 – Flow) (Fig. 16).

Results of the power analysis showed that the variable “total margin length before loading” was of .982, which means that with a total sample size of 48 there is a 98.2% chance of detecting a difference if it exists. The observed power of the variable “total margin length after loading” was of 1.000, which means that with a total sample size of 48 there is a 100% chance of detecting a difference that is really there.

DISCUSSION

This study served to test the marginal adaptation of open sandwich class II restorations with different dentine replacement materials and to assess the value of applying a three step etch and rinse adhesive system before the application of the self-adhesive restorative material Biodentine®. For this purpose, the study consisted of five experimental groups. Each material used in this study was applied according to manufacturer's instructions except in Group 4, where and adhesive layer was applied before the placement of Biodentine®.

Cavity size has a great impact on stress values and mechanical behavior of the restored tooth (Kantardžić et al. 2012). This is why large Class II cavities were selected as the experimental cavity shape to mimic clinical situations of severely damaged teeth and to maximize the influence of thermo-mechanical loading (Lin et al. 2009). The restorations realized in these cavities were subjected to the extreme loading protocol of a total of simultaneous application of 1.2 million mechanical cycles and 3000 thermal cycles, a protocol which was reported to correspond to around five years of clinical service (Sakaguchi et al. 1986). Thermomechanical loading tries to simulate the clinical fatigue process to the restorative systems, as besides chemical and enzymatic factors, fatigue is considered one of the major factors responsible for degradation of restorations' margins. The results of this study confirmed that marginal adaptation after fatigue is material dependent (Bortolotto et al. 2012).

An often-used evaluation method for assessment of marginal adaptation is scanning electron microscopy (SEM). It is a quantitative evaluation method because it measures the length of open and closed margins at a sufficient magnification over the entire tooth/restoration interface. 100% "continuous margin" means a perfect seal over the entire length of the margin evaluated. Open margins may potentially be prone to marginal staining and plaque retention which may result in secondary caries, making the evaluation method clinically relevant. A further advantage of this method is the fact that it is completely non-destructive, as it is based on gold-sputtered resin replicas fabricated on the basis of silicone impressions, which allows the evaluation of the marginal adaptation of the same sample at different time intervals, such as before and after thermomechanical loading. The scope of this method is to rapidly allow to judge on the potential of different operative techniques or materials in a standardized experimental setup before starting a clinical study which requires a very long period of time to be organized, pass the ethical committee, recruiting the patients and preparing the restorations as well as their evaluation, without mentioning the high costs of a clinical study and the impossibility of proper standardization of the clinical conditions. In addition to this, the technique is able to detect the early presence of an adhesive breakdown in the form of marginal gaps before catastrophic failures like loss of restoration's retention occurs (Bortolotto et al. 2012).

Biodentine® presented the lowest percentage of continuous margins after thermomechanical loading. A study that compared the microleakage of Biodentine® to a resin-based light cured glass-ionomer Fuji II LC in cervically lined restorations showed that the amount of microleakage of Biodentine® at margins located in dentin was 30% and the amount of microleakage at margins located in enamel was 20%. However, the teeth in this study were only thermocycled, without any mechanical loading (Raskin et al. 2012). Camilleri investigated Biodentine® and compared it to glass ionomer and resin modified glass ionomer cements in an open sandwich restoration. The study concluded that when Biodentine® used as a dentine replacement material overlayed with composite in the so-called open sandwich technique, a significant leakage occurred at the interface between Biodentine® and dentin (Camilleri 2013). On the other hand, Biodentine® performed well in another study on the open-sandwich technique: A quantitative evaluation of microleakage by glucose diffusion studied in vitro the marginal integrity of open-sandwich restorations of Biodentine® in comparison to a resin-modified glass ionomer cement, where the teeth were thermomechanically stressed, but with a low number of loading cycles. The study concluded that Biodentine® performed as well as the resin-modified glass ionomer cement which served for comparison (Koubi et al. 2012).

In another study Biodentine® was compared to a mineral trioxide aggregate (MTA) in respect to marginal adaptation in an open-sandwich situation where the samples were subjected to 3 months of aging in Phosphate Buffered Saline (PBS) buffer and to 150,000 cycles of cyclic loading. Biodentine® presented the highest percentage of continuous margins (91%) (Aggarwal et al. 2015). To our knowledge, our study is the only study which examined Biodentine® under a high number of thermo-mechanical loading cycles, which might be the main explanation for the low percentages of continuous margins of the Biodentine® groups after loading found in this experiment.

Two studies evaluated the bond strength between Biodentine® and restorative materials. The first one studied the interface between Biodentine® and overlying restorative materials by comparing the micro shear-bond-strength (μSBS) between Biodentine® and GIC and resin-modified glass-ionomer (RM-GIC). The μSBS was lowest for Biodentine®. According to this study, it was preferable to place the overlaying composite on Biodentine® after 2 weeks, to allow for adequate maturation of Biodentine® before composite placement. It was argued that the complete setting of Biodentine® will better withstand the contraction forces of the resin composite (Hashem et al. 2014). The aim of another study was to measure the shear bond strength of different adhesive systems (i.e., etch-and-rinse adhesive, two-step self-etch adhesive, and one-step self-etch adhesive systems) to Biodentine® after different time intervals (12 min and 24 h). It found that the mean bond strength values ranged from 9,1 to 19,6 MPa. Failures were adhesive, cohesive, and/or mixed. In case of lower bond strength values the failure was predominantly located between composite resin and Biodentine® (adhesive) and in case of high bond strength the failure was cohesive within Biodentine®. In addition, the study concluded that placement of composite resin with self-etch adhesive systems over Biodentine® showed the best shear bond strength (Odabaş et al. 2013). To our knowledge, these are the only two studies that studied the relationship between Biodentine® and an adhesive system. Regarding to our results, the Biodentine® group where the adhesive system was applied before the application of Biodentine® showed the lowest value of %CM before and after loading. This would mean that Biodentine® has a low compatibility with adhesive systems intended for use with composite materials and that this type of adhesive systems does not improve adhesion of Biodentine® to the dental substrate.

Modern hybrid resin composites contain very small glass filler particles together with a small fraction of nanofillers and/or nanofiller clusters, which increase the filler load, improve mechanical prosperities, and allows for polishable surfaces (Stefanski et al. 2012). Several studies showed that flowable composites can reduce microleakage (Ernst et al. 2002, Ölmez et al. 2004), while other studies do not confirm flowable composite to enhance marginal adaptation (Jain & Belcher 2000, Malmström et al. 2002, Neme et al. 2002). The explanation of the low CM% in our study with the flowable composite as dentine replacement on the cervical margin in dentin might be the low filler content of the flowable composite resin, applied in a thick layer (4 mm) which leads to higher polymerization shrinkage and low mechanical properties such as the high elasticity of the material (Ferracane 2008). However, our results are in contrast to other studies (Jain & Belcher 2000, Malmström et al. 2002, Neme et al. 2002) with more favorable results but the difference might be explained by the fact that these studies did not apply a high number of thermomechanical cycles.

No significant differences were observed between the three best groups (group 1 - Comp, group 2 – Flow and group 3 Bulk) before the thermomechanical loading and the results were quite low in absolute values. As it is known that the polymerization shrinkage stress may cause deformation of the cusps, debonding or enamel cracks resulting in microleakage, postoperative sensitivity and secondary caries (Ferracane 2008, Park et al. 2008), application of the composite in one bulk layer may explain this wide of variation. Beside this, a recent study evaluated the effectiveness of polymerization of three different types of composite resin. It concluded that composite resins containing nano-particles shows more variations in degree of conversion and microhardness (Jafarzadeh 2015). Another explanation for the wide variations in the results may be found in another recent study which showed that the degree of conversion (DC) of conventional composite resin and bulk-fill composites varied depending on the stratification technique.

No significant difference in DC values was observed when classic restorative composite resin was placed incrementally (Fronza et al. 2015). This result was expected by the Fronza et al., because each increment received the same irradiance as that of the entire bulk-filled restorations. Furthermore, Herculite™ Ultra has a high filler content, which results in increased light scattering, Therefore, fewer photons may have reached deeper layers when the bulk-fill technique was used, explaining why lower DC value was obtained at the deepest depth with this type of composite (Fronza et al. 2015).

Taking together, despite very different mechanical properties, the results of percentages of continuous margins for the total marginal length of the three resin-based dentin replacement materials were not significantly different both before and after loading, confirming the results of another two studies where the microleakage and the marginal adaptation of a highly filled composites and SureFil™ SDR were similar (Scotti et al. 2014, Orłowski et al. 2015). This shows that resin composite materials are still the reference in terms of marginal adaptation, especially in class II restorations. Moreover, this study showed that despite its advantage in terms of biocompatibility, Biodentine® does not appear to be a suitable dentin replacement in open sandwich restorations due to its low quality of marginal adaptation, especially after loading. These results need to be taken while considering the limitations of this in-vitro study, which were the use of extracted teeth, and the application of artificial loads that do not necessarily mimic the exact natural forces in the mouth.

CONCLUSIONS

Based on the results and within the limitations of this *in vitro* study, the following conclusions can be drawn: Herculite™ Ultra and SureFil™ SDR performed best of all the groups tested as dentin replacement layer in open sandwich restorations covered by a layer of Herculite™ Ultra. The group where SureFil™ SDR was used as dentin replacement layer showed better marginal adaptation than the group with the flowable resin composite Premise™ Flowable as dentin replacement. Biodentine® as dentin replacement layer in open sandwich restorations showed very low marginal adaptation. A three step etch and rinse adhesive system did not improve the marginal adaptation of Biodentine® in a significant way, on the contrary, the scores of continuous margins were even lower, which leads to the conclusion that

Biodentine® does not appear compatible with an adhesive system for methacrylate based composites.

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TABLES

TABLE 1: Materials used in the study.

Material	**Product**	**Composition**	**Lot No.**	**Manufacturer**
Restorative Composite	Herculite™ Ultra	Bis-GMA, TEGDMA, prepolymerized fillers, SiO_2, barium glass fillers, TiO_2, pigments	4779204	Kerr
Dentin replacement	Premise™ Flow	Bis-EMA, TEGDMA, silica nano-filler	4776472	Kerr
	SureFil™ SDR	Barium-alumino-fluoro-borosilicate glass, strontium alumino-fluoro-silicate glass, EBPADMA, TEGDMA, CQ photoinitiator, photoaccelerator, BHT, UV stabilizer, titanium dioxide, iron oxide pigments, fluorescing agent	130310	Dentsply
	Biodentine®	Tricalcium silicate powder, aqueous calcium chloride solution, excipients	B08323	Septodont
Adhesive system	Optibond™ FL	Gel etchant: 37.5% H_3PO_4, water, and fumed silica Primer: HEMA, GPDM, MMEP, water, ethanol, photoinitiator (CQ), and BHT Adhesive: Bis-GMA, HEMA, GPDM, GDMA, photoinitiator (CQ), SiO_2, bariumaluminoborosilicate, Na_2SiF_6)	4905400	Kerr

FIGURES

FIGURE 1: Photograph of the metallic tube inserted into the root of the teeth to allow dentinal fluid simulation.

FIGURE 2: Photograph of the setup used to connect the serum reservoir to the tooth, using a 1.4 mm tube.

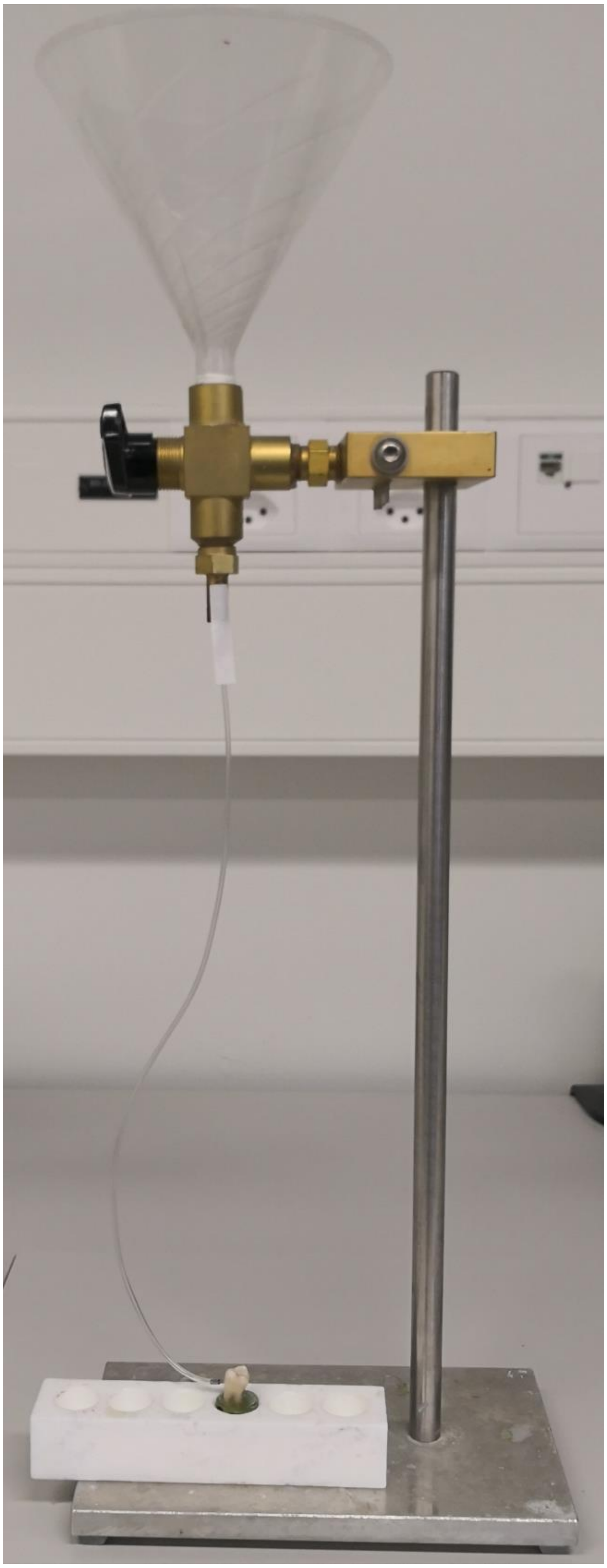

FIGURE 3: Photograph of the handpiece holder that allows to perform standardized cavities by restricting the bur's position.

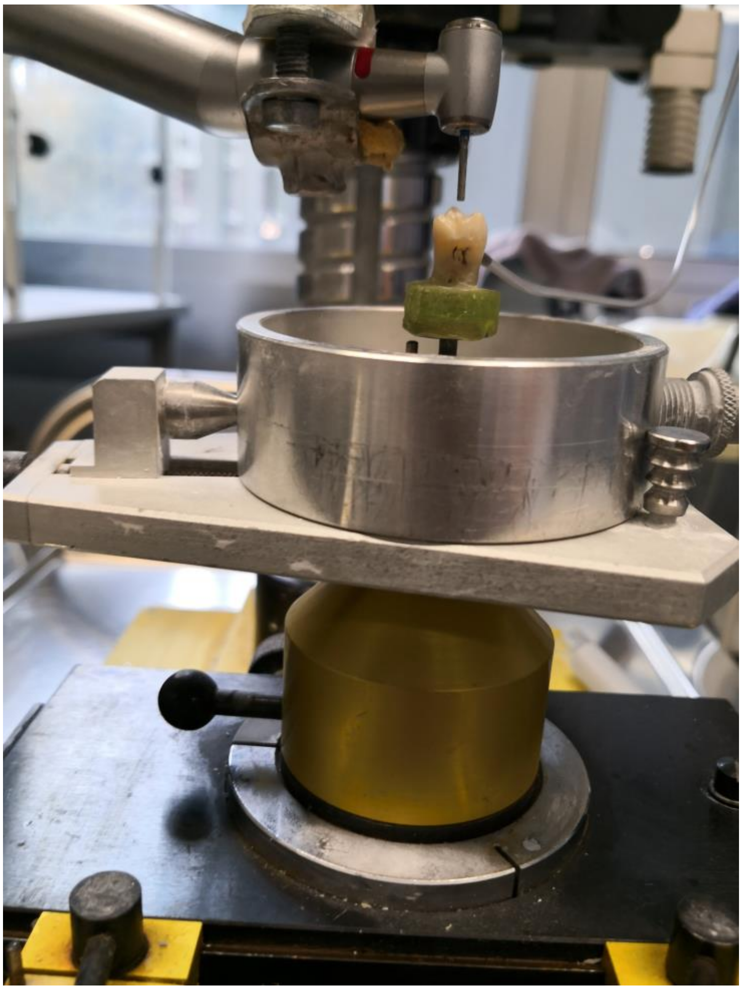

FIGURE 4: Schematic representation of the proximal view of the five groups of the study.

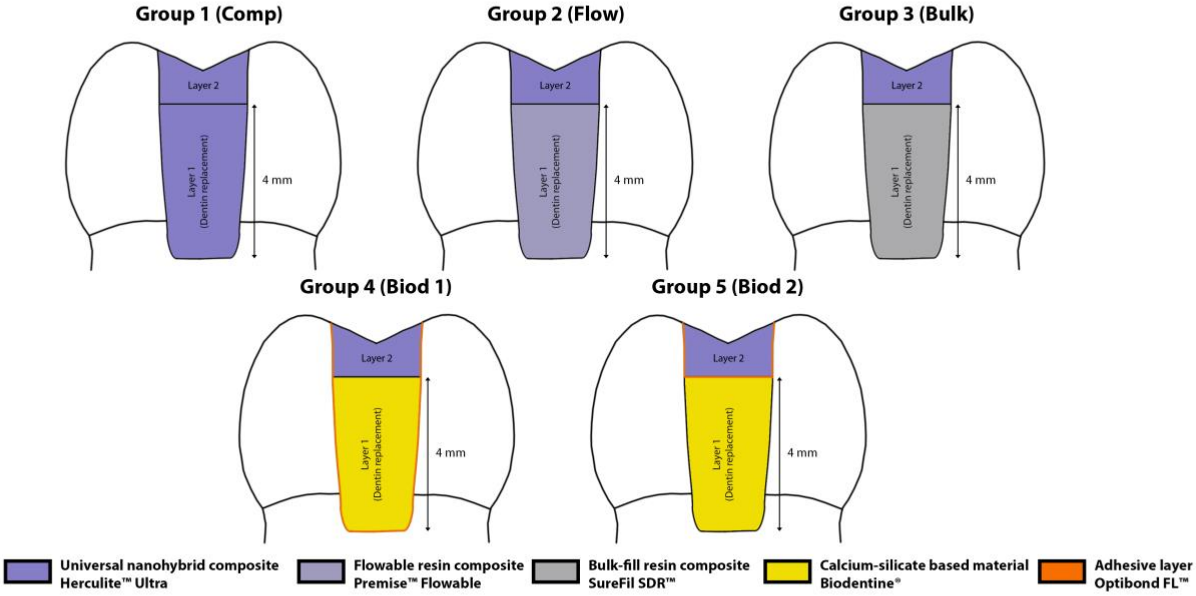

FIGURE 5: Photograph of the thermo-mechanical cyclic testing cell.

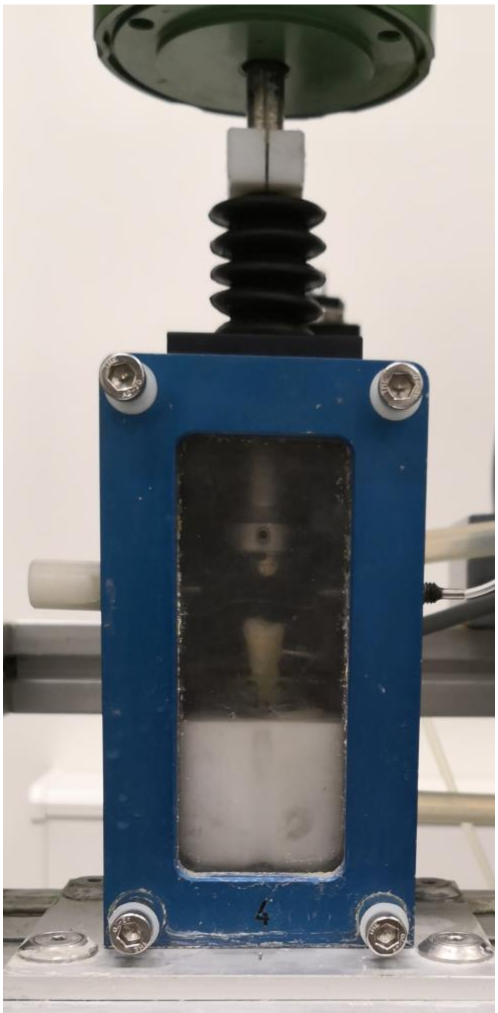

FIGURE 6: Photograph of a gold-coated replica.

FIGURE 7: Microphotographs representing a) a continuous marginal interface, and b) non-continuous marginal portions.

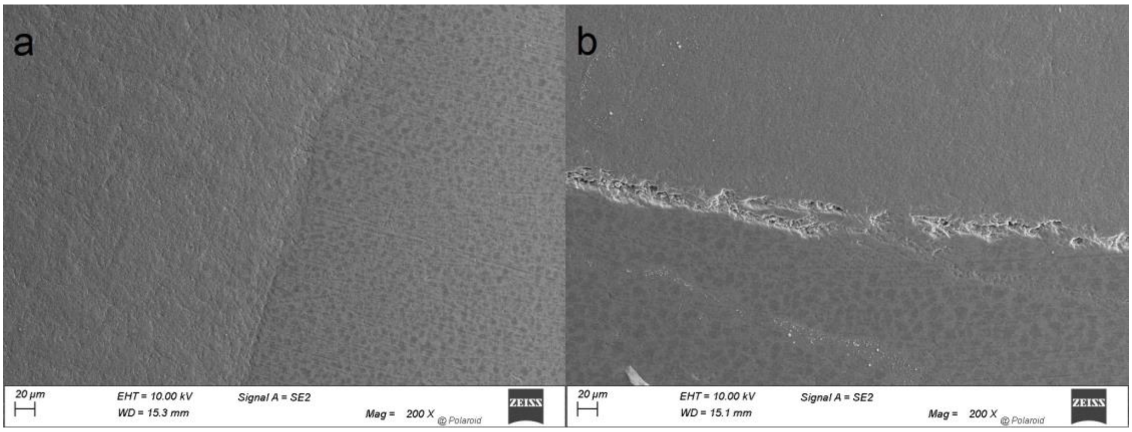

FIGURE 8: Schematic representation of the analyzed regions of interest at the marginal level. Green represents the occlusal margin, blue the axial margin, and red the cervical margin. The total length of the margin (TML) corresponds to the sum of the three previously described regions.

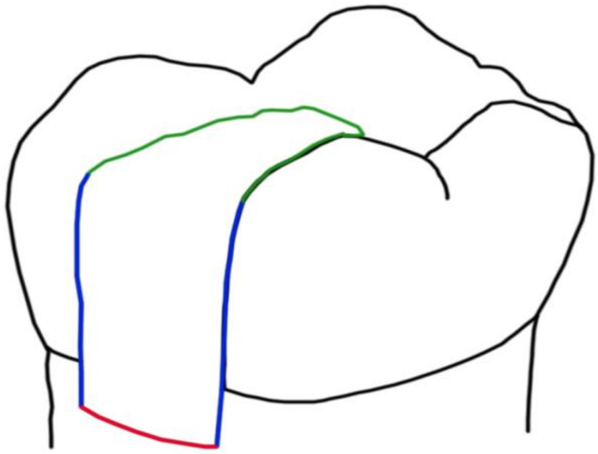

FIGURE 9: Box plot of the results of percentages of Continuous Margin (CM) at the total margin length (TML) before the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

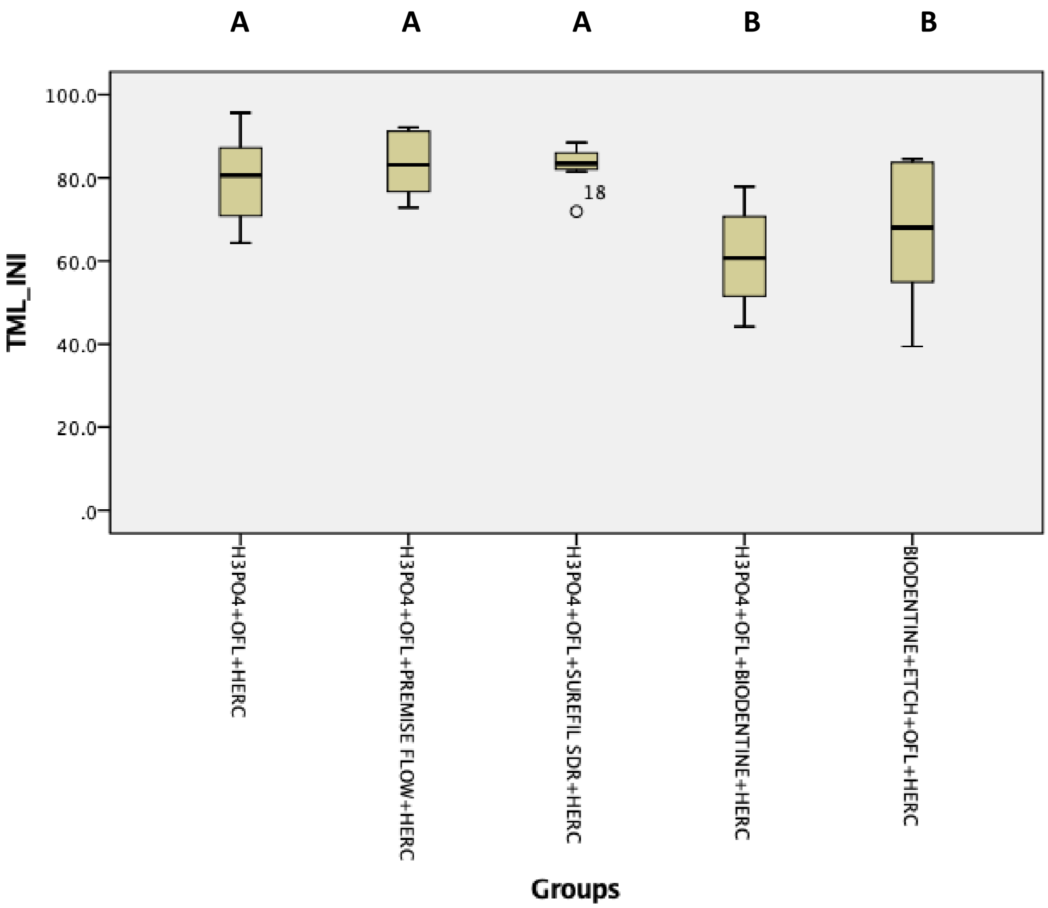

FIGURE 10: Box plot of the results of percentages of Continuous Margin (CM) at the total margin length (TML) after the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

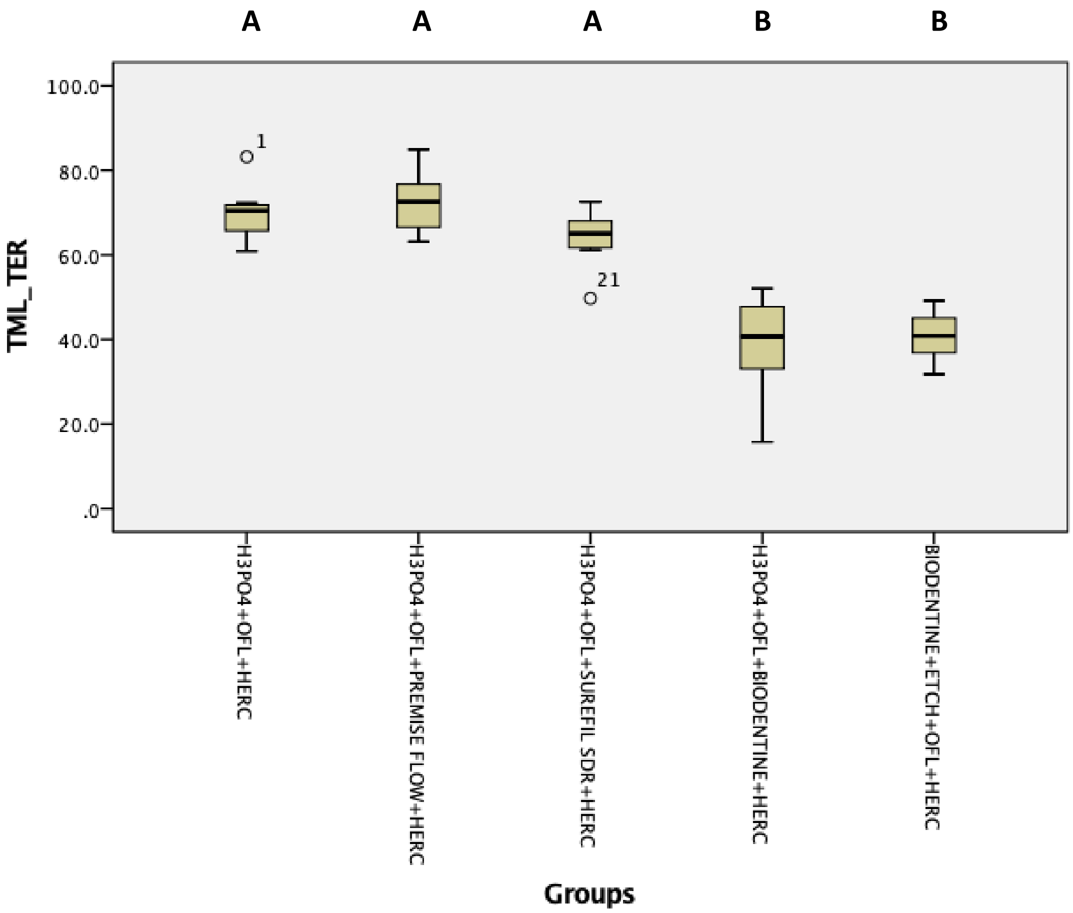

FIGURE 11: Box plot of the results of percentages of Continuous Margin (%CM) at the occlusal margins in enamel (region a) before the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50^{th} percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25^{th} percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75^{th} percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

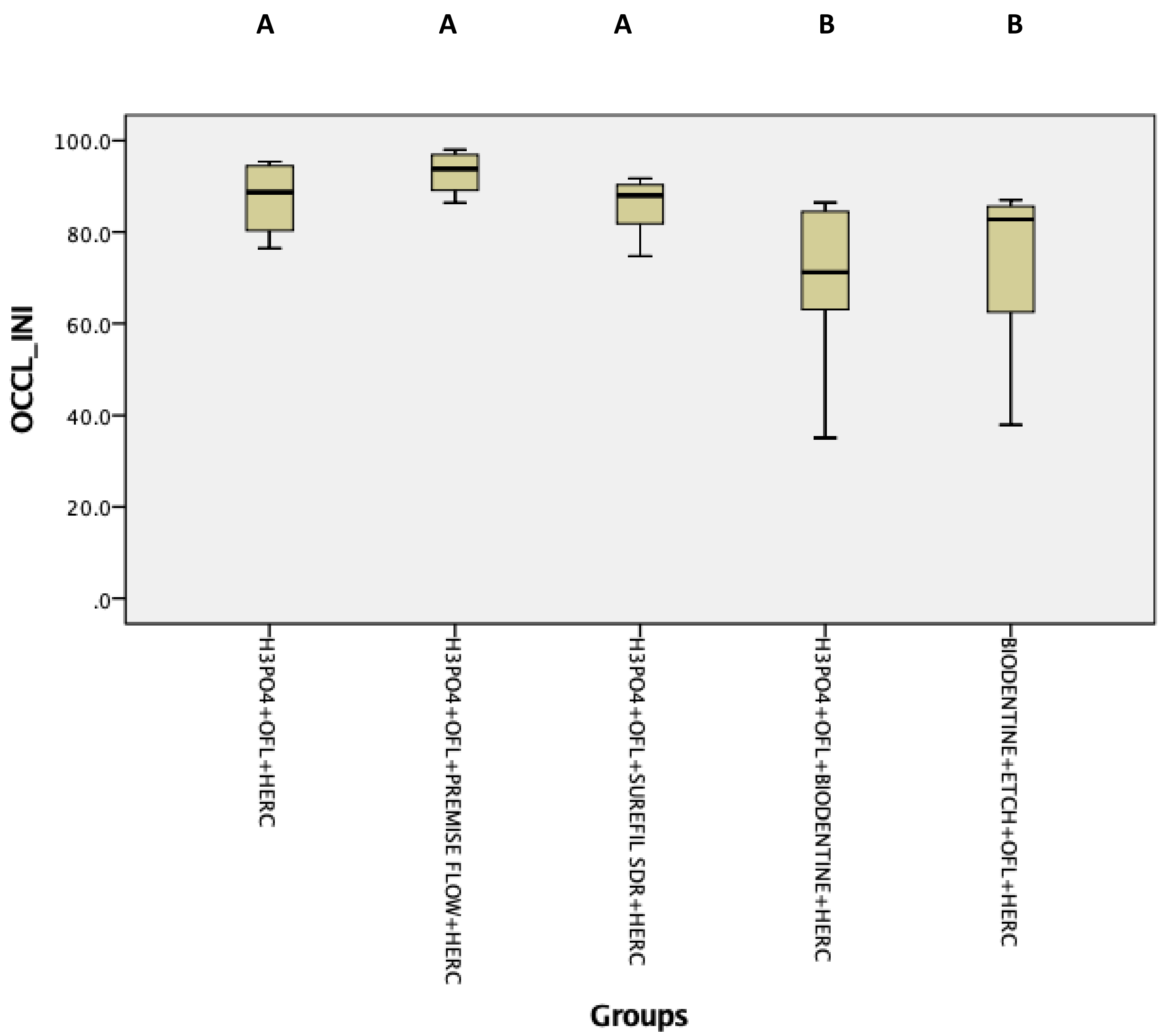

FIGURE 12: Box plot of the results of percentages of Continuous Margin (CM) at the occlusal margins in enamel (region a) after the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

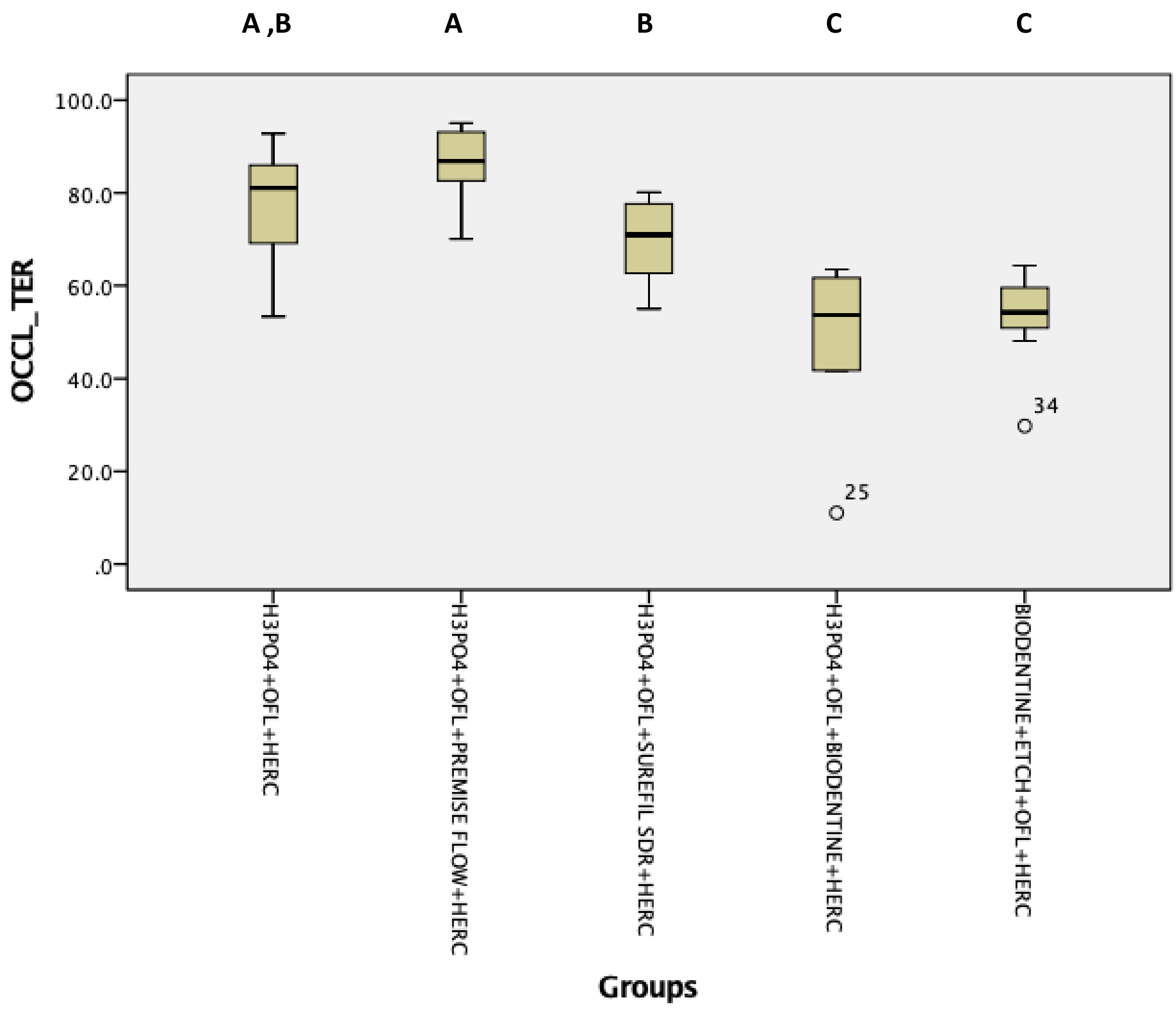

FIGURE 13: Box plot of the results of percentages of Continuous Margin (CM) at the axial margins in enamel of the box with cervical margin in dentin (region b) before the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

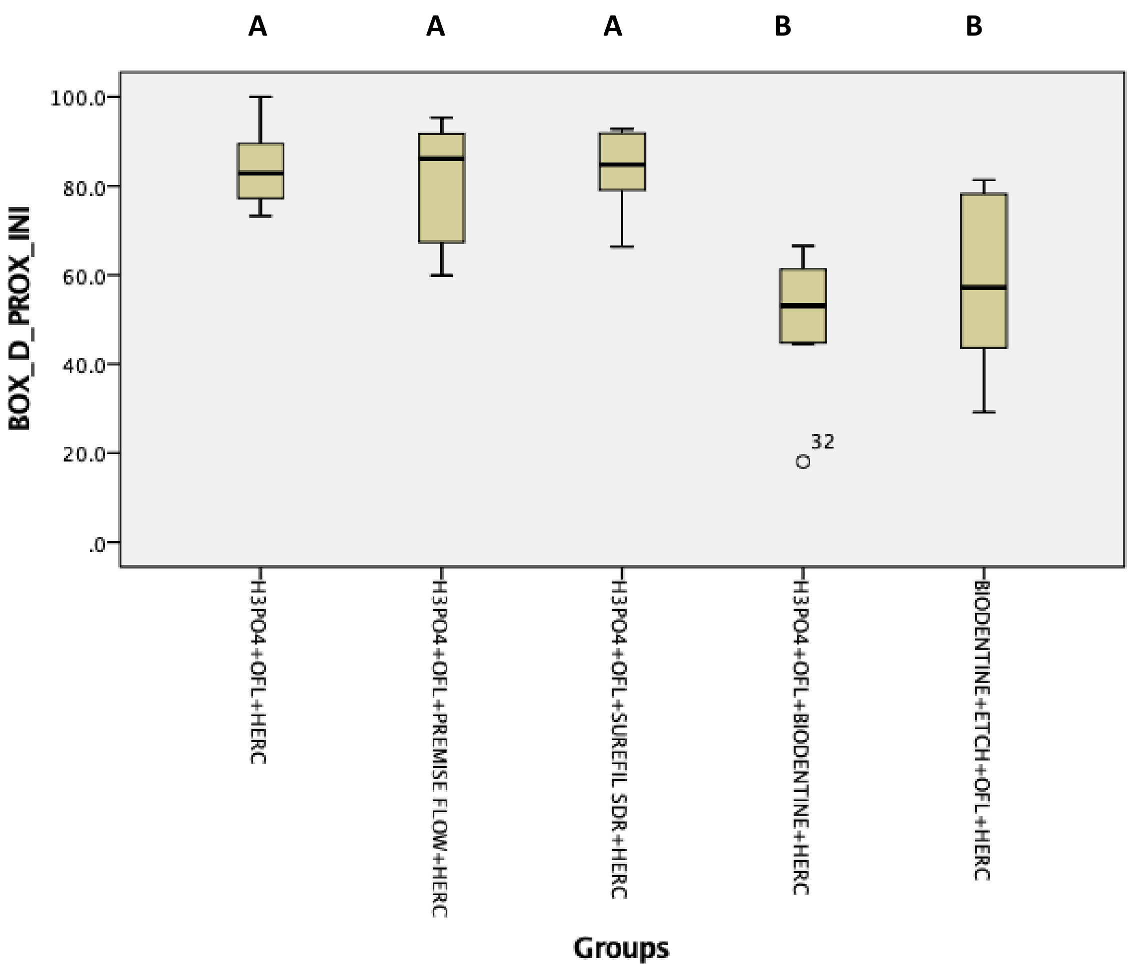

FIGURE 14: Box plot of the results of percentages of Continuous Margin (CM) at the axial margins in enamel of the box with cervical margin in dentin (region b) after the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

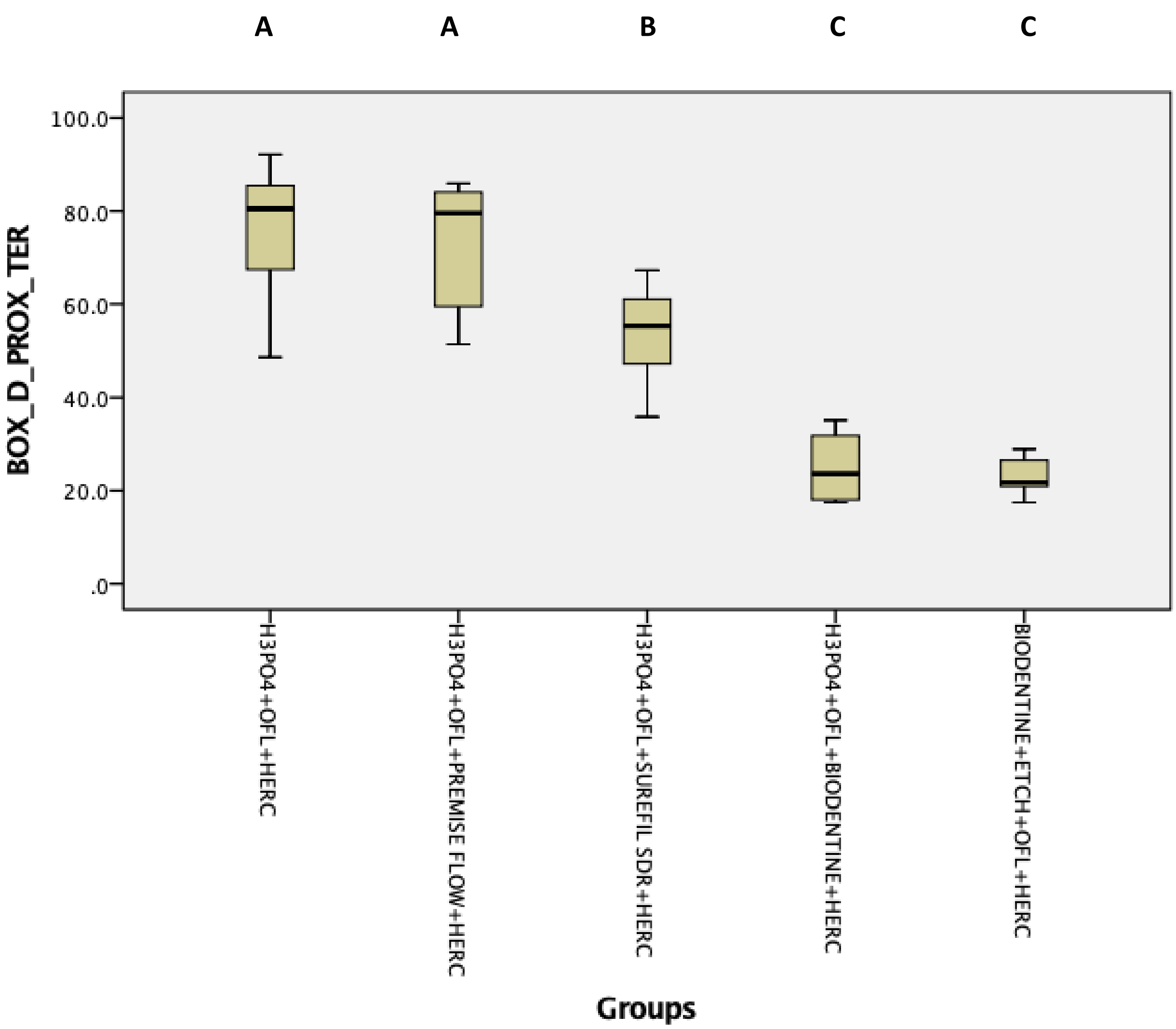

FIGURE 15: Box plot of the results of percentages of Continuous Margin (CM) at the cervical margins in dentin (region c) before the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.

B **B** **A** **B** **B**

FIGURE 16: Box plot of the results of percentages of Continuous Margin (CM) at the cervical margins in dentin (region c) after the fatigue test. Groups with different capital letters are significantly different ($p < 0.05$). The median or 50th percentile, i.e. the values below which 50% of the results fall, is the dark line in the middle of the box. The 25th percentile, i.e. the values below which 25% of the results fall, is represented by the bottom of the box and the 75th percentile, i.e. the values below which 75% of the results fall, is represented by the top of the box. The T-bars extending from the boxes are called whiskers, the horizontal lines (t) represent the minimum and maximum values excluding outliers. The circles are extreme values and are called mild outliers, they are defined as values that do not fall in the whiskers. The asterisks are extreme outliers, they appear in the graph when there are values more than 3 times the height of the boxes.