Evaluation of interface between tooth and dental composite resin with monocalcium phosphate MCP

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ABSTRACT

Sealing ability of dental material could determine long-term clinical outcomes of restoration. Monocalcium phosphate (MCP) added in dental composites promotes swelling and precipitation of apatite at the tooth restoration interface. The aim of this study was to assess whether these features can improve sealing capability at the tooth-material interface when no bonding agent was employed. A bonded composite was used as control. Cavities; 3 mm in diameter and 2 mm in depth, were prepared in the middle of buccal surfaces of sound premolars. Fifteen samples (N=5/group) were randomly assigned for restoration either in Group I- conventional composite (Filtek Z350XT;Adper Single Bond 2), Group II- composite with MCP (Renewal MI), and Group III-composite-MCP aged in artificial saliva for 28 days (aged Renewal MI). After restoration and thermocycling 5,000 times between 5 and 55 °C, teeth were sectioned and gap formation at the tooth-restoration interface were assessed using scanning electron microscopy (SEM). Whereas all samples restored with Filtek Z350 XT with adhesive system; Adper Single Bond 2, had no gap at the material / enamel interface, both aged and unaged Renewal MI formed gaps. Aged Renewal MI, however, showed smaller gaps. Furthermore, in this group layer of element was observed intermittently at the interface. The results that aged Renewal MI formed narrower interfacial gap suggest that MCP added in dental composite, when contact with artificial saliva, might improve sealing of restoration by material expansion and precipitation.

Keywords: Dental Composite, Mono Calcium Phosphate, Self-Adhesive Composite, Micro-Gap, Scanning Electron Microscopy

1. Introduction

Resin composites have become the first choice for direct anterior and posterior restorations. The popularity is derived from their aesthetic appearance and the physical properties being superior to other tooth-coloured restorative materials. The immediate clinical outcomes are well-accepted by dentists and patients. Additionally, adhesive systems allow a minimally invasive tooth preparation technique (AlHumaid, Al, & ElEmbaby, 2018; Mahajan et al., 2015). Nevertheless, their treatment outcomes are known to be highly sensitive to restorative techniques. Moisture contamination during surface preparation could compromise the integrity of the hybrid layer (Niu et al., 2014; Thompson et al., 2013).

Polymerization shrinkage remains a major disadvantage of composite resins (Lopes, Costa, Consani, Gonini, & Sinhoreti, 2009). Incremental application is recommended as one of the techniques to reduce the shrinkage, particularly, when used to restore a large cavity (Chandrasekhar, Rudrapati, Badami, & Tummala, 2017; Donly & Jensen, 1986). However, it is time consuming. Total etch systems, consisting of acid etching and bonding, either separately or combined, requires effective isolation of the working area from saliva contamination. When applied appropriately, all the available adhesive systems construct a good micromechanical retention to the enamel (Tezvergil-Mutluay, Pashley, & Mutluay, 2015).

Unlike the enamel, dentin is richer in protein and water. Dentine matrix is composed of collagenous and non-collagenous proteins that form a scaffold for mineral deposition. Acid etching dissolves the inorganic components and provides space for resin tags. It can also, however, initiate release of inherent protease and collagenase enzymes that can cleave the organic matrix and gradually cause degradation of hybrid layer (Niu et al., 2014). Penetration of adhesive into the exposed collagen and formation of the interfacial layer require optimal dehydration that is difficult to achieve in the water rich dentine. Due to its nature, the effectiveness of the resultant hybrid adhesive / dentine layer has mostly been compromised at the dentine surface. Leakage at the interface, caused by polymerization shrinkage and degradation of the hybrid layers over time, leads to a major complication of secondary caries (Spencer et al., 2010).

Improvement of composite materials has focused around enabling reduction in clinical procedures and facilitating material handling. Examples include production of self-adhering dental composite (AlHumaid et al., 2018; Jordehi, Shahabi, & Akbari, 2019; Rengo et al., 2012) that require no separate bonding step as well as flowable composites with reduced filler load that can be directly "injected" into cavities (Ozel Bektas, Eren, Akin, & Akin, 2013; Vichi, Goracci, & Ferrari, 2010). It has been shown to provide better adaptation to the internal cavity wall, reduced internal voids and post-operative sensitivity when compared with conventional composites (Bhatti, 2019).

Most commercially available composite materials have limited bioactive abilities, such as anti-microbial action or remineralization induction that help preventing or repairing recurrent caries. Filling the tooth with bioactive composites, not only restores the damaged structure, but also induces repairing process by the biological response of vital tissue. In vulnerable patients with poor plaque control, such as patients with special needs and young children, conventional composite restoration may not be the material of choice.

Recently, a semi-flowable dental composite, named “Renewal MI”, with monocalcium phosphate (MCP) in the filler has been formulated. Monocalcium phosphate has been widely used in the food industry and agriculture. In the biomedical field, MCP has been shown to increase bone regeneration (Huan & Chang, 2009). In dental composites, it has been demonstrated to promote swelling and precipitation of apatite at the tooth restoration interface when contacted with simulated body fluid (Aljabo et al., 2015; Kangwankai et al., 2017). Furthermore, MCP reacts with absorbed water. This can increase material expansion and thus compensate the polymerization shrinkage (Kangwankai et al., 2017).

Renewal MI has urethane dimethacrylate (UDMA) as its base monomer in addition to high molecular weight polypropylene glycol dimethacrylate (PPGDMA) as diluent. This diluent improves material flow (Kangwankai et al., 2017), whilst its high molecular weight lowers polymerisation shrinkage and enhances monomer cytocompatibility. Additionally, low levels of 4-methacryloyloxyethyl trimellitate anhydride (4-META) that can react with water to form 2 carboxylic acid groups and bond to calcium ions in hydroxyapatite has been dissolved in these monomers (Ben Nuba, 2016; Kangwankai et al., 2017; Panpisut et al., 2016; Paulo, da Rocha Svizero, & Carrilho, 2018).

Renewal MI also has low levels of the FDA approved food preservative, ε-poly-L-lysine (PLS), added as part of the fillers. This compound is known to have a wide anti-microbial spectrum but low toxicity to human cells (Yoshida & Nagasawa, 2003). Due to the positive charge, PLS can promote multiple crosslinking ionic interactions between MCP, hydrolysed 4META, collagen and apatite (Shih, Shen, & Van, 2006). The multiple component interactions have the potential to enable bonding to tooth structure, without using etching and bonding adhesives (Kangwankai et al., 2017; Panpisut et al., 2016). Prior studies have demonstrated the ability of combined MCP and PLS to promote material expansion and apatite precipitation when set composites are placed in simulated body fluid. MCP and PLS can reduce physical properties as a result of enhancing water sorption. Restriction of their levels, however, enables such properties to be maintained within the range of other commercial composites (Aljabo et al., 2015; Kangwankai et al., 2017).

The propose of this in vitro study was to characterize the interfacial feature of Renewal MI, employed without etch and bond system, under conditions, with or without immersion in artificial saliva for 28 days.

2. Objectives of the study

The objective of the study was to characterize the morphology of tooth-material interface of Renewal MI in the condition with or without aged in artificial saliva, in comparison with the commonly used bonded composite.

3. Materials and methods

This in *vitro* study was approved by the Khon Kaen University Ethics Committee for Human Research, Thailand (Protocol Number HE622134).

Sample preparation

Fifteen sound premolars, stored in 0.1% thymol solution not more than 60 days, were included in the study. A cavity of 3-mm width, 3-mm length and 2-mm depth was prepared in the middle of buccal surfaces of each tooth using a diamond cylinder bur in a high-speed hand piece with air-water coolant. The depths of the cavity were confirmed with a periodontal probe.

Restorative procedures

The samples were randomized into three experimental groups according to the restorative material (5 teeth per group). Table 1 shows material compositions. After filling the cavities, samples were kept in a 100% relative humidity chamber at 37°C overnight before finishing with Sof-Lex pop-on discs (3M ESPE, St. Paul, MN, USA) and until further analysis.

Table 1: Composition of the studied materials

Materials	Etch-rinse adhesive system	Filtek™ Z350 XT	Renewal MI
Composition	Etchant: 35% phosphoric acid, Adhesive: Bis-GMA, HEMA, silane-treated silica, ethanol, glycerol 1,3-dimethacrylate, diurethane dimethacrylate, copolymer of acrylic and itaconic acids	Methaacrylate resin monomers, Bis-GMA, TEGDMA, Bis-EMA, photoinitiator, Filler	UDMA, PPGDMA, 4-META, silica, camphorquinone, silane treated aluminosilicate glasses, MCP, ε-poly-L-lysine
Manufacturer	3M ESPE, St Paul, MN, USA	3M ESPE, St Paul, MN, USA	Schottlander, Letchworth, UK
Abbreviations: Bis-GMA: Bisphenol A glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; TEGDMA: Triethylene glycol dimethacrylate; UDMA: Urethane dimethacrylate; PPGDMA: Polypropylene glycol dimethacrylate; 4-META: 4-methacryloxyethyl trimellitic anhydride; MCP: mono-calcium phosphate			

In Group I: Cavities were rinsed and air dried. 35% phosphoric acid (Scotchbond™ Etchant Phosphoric Acid; 3M ESPE, St. Paul, MN, USA) was added for 15s rinsed and air blown slightly to give moist dentine. Adhesive (Adper Single Bond 2; 3M ESPE, St Paul, MN, USA) was applied for 15s, dried for 5s then light cured for 20s as per manufacturer's instructions. The packable composite (Filtek™ Z350 XT; 3M ESPE, St. Paul, MN, USA) was then placed and LED light (light intensity of 1100 mW/cm^2) cured for 40s (Demi PLUS, Kerr, Washington, DC, USA). The tip-to-restoration distance was 1 mm.

In Group II: Cavities were rinsed and air blown slightly to obtain a moist dentine condition, prior to placing the semi-flowable dental composite with MCP (Renewal MI, Schottlander, Letchworth, UK). The material was provided in a ready-to-use single capsule to be mounted in the dispenser (Figure 1). The light cure time recommended was 40s.

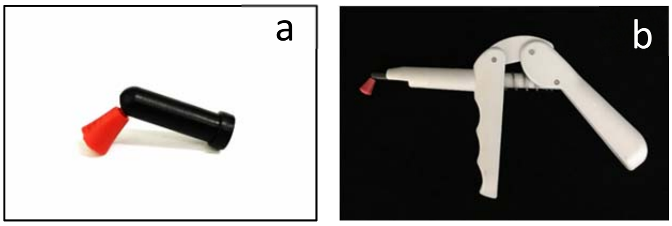

Figure 1. (a) Renewal MI capsule, (b) Renewal MI capsule mounted in the dispensing gun

In Group III: Restorative procedures were similar to those in Group II. After finishing , samples were immersed in artificial saliva (AS) of volume 10 ml/tooth at 37°C for 28 days (BS ISO23317:2012)(BS-ISO, 2012; Panpisut et al., 2016). The artificial saliva was composed of KCL 0.625 g/L, $MgCl_2$ •$6H_2O$ 0.059 g/L, $CaCl_2$ 0.166 g/L, KH_2PO_4 0.326 g/L, K_2HPO_4 0.804 g/L, Sodium benzoate 2 g/L and Sodium Carboxy methyl Cellulose 10g/L in 1000 mL of deionized water. 1 molar solution of Hydrochloric acid (HCl) was used to adjust pH level to 7. (Scatena, Galafassi, Gomes-Silva, Borsatto, & Serra, 2014)

Thermocycling and tooth sectioning

All restored teeth were subjected to a thermocycling process for 5,000 cycles; between 5°C and 55°C with 30 seconds for a dwell time (ISO 11405:2015) (ISO, 2015; Pisuttiwong & Leevailoj, 2017) and 10s for a transfer time. (Gupta, Verma, & Trivedi, 2011) The tooth roots were embedded in 3/4-inch PVC tube using acrylic resin and the crowns were coated with epoxy resin in order to prevent restoration dislodgement while cutting (Kitsahawong, Seminario, Pungchanchaikul, Rattanacharoenthum, & Pitiphat, 2015). All samples were cut with water coolant in the buccolingual direction through the center of the restoration using cutting machine (Mecatome T180, Brié-et-Angonnes, France); section M (mesial) and D (distal) (Figure 2). Section M of each tooth was used for SEM analysis.

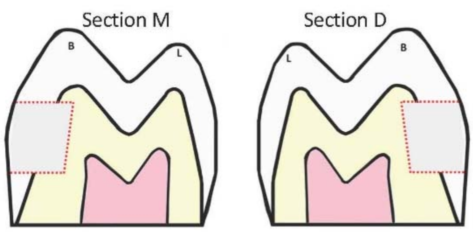

Figure 2. Diagram of tooth sample, halved into mesial (M) and distal (D) sections.

Scanning electron microscope analysis

Section M of each tooth was used for SEM analysis. Analysis surface of each tooth were polished using polishing machine (Ecomet®3, Buehler, USA) with sand papers NO. 1000,1500,2000 and 4000, and diamond suspension (Buehler Ltd., USA) under running water for 1 min each. All samples were then dried in a NORTHMAN desiccator cabinet for 4 hours, and sputtered coated with gold. Each specimen was evaluated using scanning electron microscopy (S-3000N Scanning Electron Microscope 50E5126 version 09-03-2122, Hitachi S-300N, Osaka, Japan). The examiner was blinded for the type of restoration during analysis.

4. Results

Morphology of the tooth-restoration interfaces were different among groups, which can be clearly identified in the micrographs. Examination was performed at the enamel and dentine interfaces.

Enamel interface (Figure 3):

The interface of group I was completely sealed (Fig. 3a-b). In group II, the micro-gap was apparent along the interface and it was smaller than 5 µm (Fig. 3c-d). In group III, the micro-gap was visible along the interface and smaller than in group II (Fig. 3e-f).

Dentine interface (Figure 4):

Interfacial gaps were found in group I. In Group II, the gaps which approximately 30-40 µm width were observed along the surface (Fig. 4c-d). In Group III, the micro-gaps which less than 20 µm were detected and layers of particles were found in the gap. Moreover, the imprints of spherical polylysine particles can be observed (Fig. 4c).

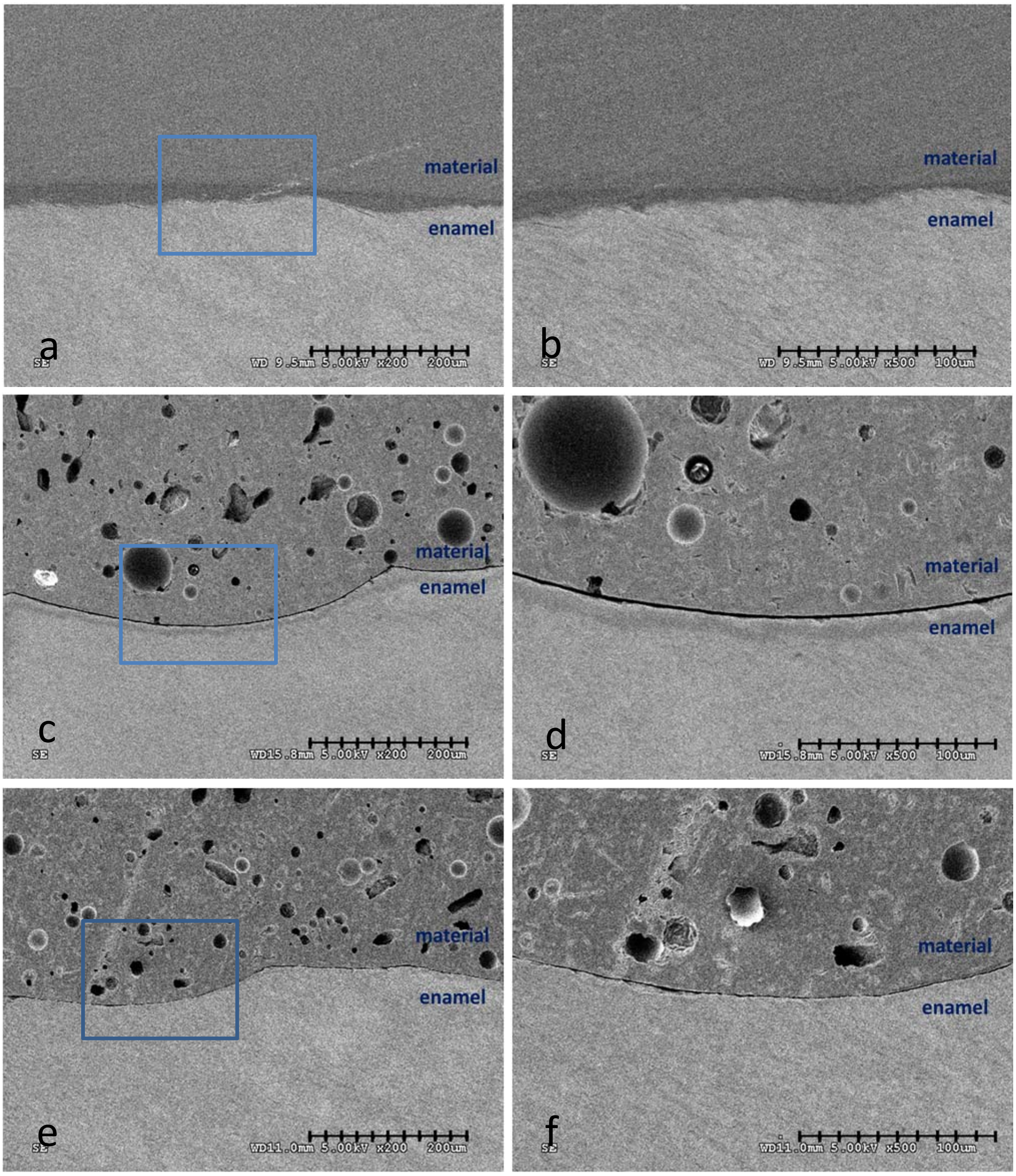

Figure 3. SEM images of enamel interfaces; a,c,e at 200X magnification, and b,d, f at 500X magnification of areas corresponding to blue islets. Group I was no gap formation along the interface (a,b). Group II showed gap formation along the interface(c,d). Group III presented smaller interfacial gap formation as compared to Group II (e,f).

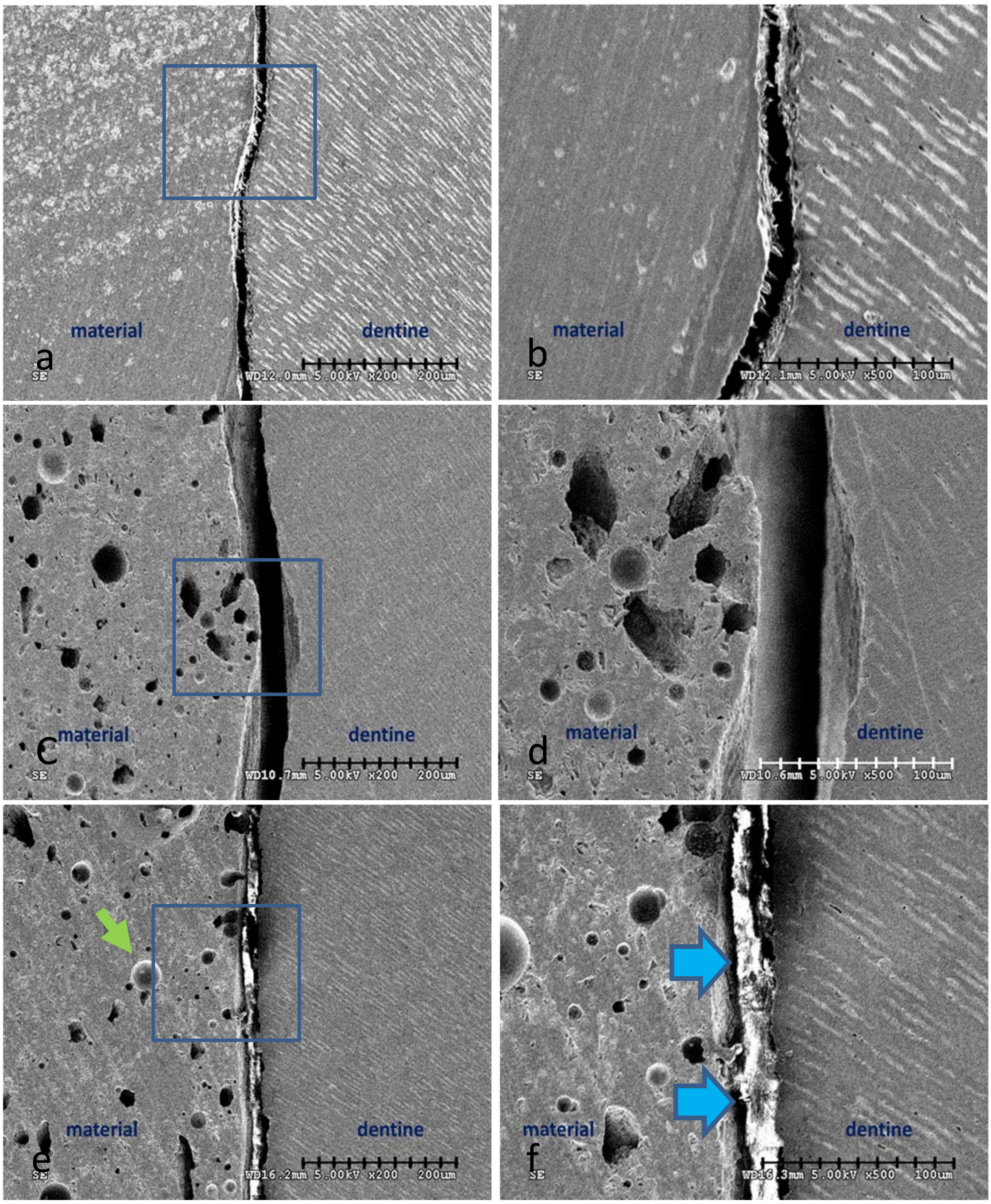

Figure 4. SEM images of dentine interfaces; a,c,e, at 200X magnification, and b,d,f, at 500X magnification of areas corresponding to blue islets. Group I showed the gap formation at approximately 10-15 µm along the surface (a,b) Group II exhibited the gap formation approximately 30-40 um (c,d). Group III presented the gap formation approximately 20 µm (e,f) and various amount of particles (blue arrows).

5. Discussion

The interface of dentine-restorative material is a crucial factor to determine the success, and failure, of the restoration. In the composite resin with adhesive system, micro-mechanics created in the etched enamel layer show a promising clinical outcome. However, the hybrid layer that initially retained the restoration to dentine, has proven to be the weakness for its long-term survival (Betancourt, Baldion, & Castellanos, 2019; Kumar, Tekriwal, Rajkumar, Gupta, & Rastogi, 2016; Spencer et al., 2010). Denuding dentinal collagen bundles by acid etching and incomplete penetration of the adhesives into etched dentine have been inevitable results of the conventional adhesive systems. With time, these will enhance physical and biological challenges initiating the deterioration process of the hybrid layer (Niu et al., 2014). Extensive studies have attempted the modification of properties of bonding materials to dentine (Betancourt et al., 2019; Thompson et al., 2013). Omitting the surface preparation step sounds appealing, not only to reduce the chair-time, but also to avoid harming the integrity of dentinal structure. However, without hybrid layer, some concerns could be raised regarding the adaptation of composite resin and its bond strength. Direct examination of the interface under scanning electron microscope could demonstrate the characteristic on how the materials performed at the interface as well as could serve as important preliminary data on the performance of material.

In the present *in vitro* study, all samples were subjected to a thermocycling challenge imitating the degradation of material in the oral cavity approximately for 6 months. The conventional composite with an etch-rinse adhesive system, selected as the control, showed the best marginal sealing with no gap formation at the enamel interface. One of the purposes of this study was to examine the effect of water sorption of the MCP containing composite, all 15 samples in this study has been kept in 100% relative humidity chamber after the thermocycling, instead of immersion in storage solution. This might cause the dentine to have dried out and later became over-dehydrated during the preparation process for SEM, whereas the enamel seemed to be unaffected. The detachment seen at the dentine interface might be a result of dentine shrinkage during the mentioned process (Zhang, Mao, Lu, Romberg, & Arola, 2009). Remnants of resin tags seen on the material side might indicate that bonding had been achieved through the etch-rinse adhesive system. In unaged Renewal MI samples, the irregular edge on the dentine, caused by the diamond bur during cavity preparation mirrored that of the restoration surface. This might indicate a good adaptation of the material onto the cavity floor had been achieved. This false positive should be taken into consideration for further tests.

Previous studies have reported that dental composite with MCP could expand and induce precipitation at the surface when immersed in simulated body fluid (SBF), over a period of time (Kangwankai et al., 2017; Panpisut et al., 2016). Artificial saliva in the present study was prepared with similar composition to the SBF used in previous studies (Aljabo et al., 2015; Panpisut et al., 2016). The un-aged composite with MCP had been in contact with water during 5-day-process of thermocycling, however, micro-gap formation could still be observed along the interface between restoration and tooth structures. After aging in artificial saliva for 28 days, the dental composite with MCP, in the condition without bonding system, has become better adapted to both enamel and dentine.

As no extra-adhesive system was used for Renewal MI with MCP, it was anticipated that there would be a transient micro-gap at the interface, at the initial stage following restoration. It has been reported in a retrospective study that the average width of the gap between occlusal margin and amalgam restoration at 187 µm could determine the occurrence of secondary caries (Hodges, Mangum, & Ward, 1995). Micro-gap seen in the SEM micrographs, particularly at the enamel, appeared to be smaller. In the previous studies, it was hypothesized that micro-gaps of the tested composite could subsequently be compensated by expansion of the material, by formation of the low density apatite, and gradually, by chemical bonding of the self-adhesive properties to dental tissue (Aljabo et al., 2015; Kangwankai et al., 2017; Panpisut et al., 2016). SEM analysis in this study could support the previous finding on material expansion, through the smaller interfacial gap formation of the aged Renewal MI. Hydrophilic components including monocalcium phosphate and ε-poly-L-lysine have been selected for the formula, in part, to promote water intake and mineralization of restoration (Aljabo et al., 2015; Kangwankai et al., 2017; Panpisut et al., 2016). It has been showed that the increased mass and volume were due to water-absorption and that the absorption rate of the tested material was high and reached a plateau between 1-3 months (Ozel Bektas et al., 2013). Material's mass and volume changes were relatively comparable to the available commercial composites and to the bioinductive composite with similar ratio of added Ca-P fillers (Aljabo et al., 2015; Kangwankai et al., 2017).

Layers of particle were also found along the interface. It is possible that these elements are calcium deficient apatite that has been shown in previous studies to form on the surface of the aged composite with MCP and adjacent dentine (Aljabo et al., 2015; Kangwankai et al., 2017). Further analysis, such as Energy Dispersive X-ray Spectroscopy (EDS) could be performed to verify these elements. Previous studies on this composite containing MCP reported the precipitation layer of the bioapatite (approximately 15 µm) at material surface after 4-week immersion in simulated body fluid (Aljabo et al., 2015; Kangwankai et al., 2017). It has been demonstrated that, with time, the reactive calcium phosphate (Ca-P) fillers in the composite dissociated in the presence of water and precipitated as dicalcium phosphate and phosphoric acid. When the later makes contact with ions provided by the artificial saliva this can promote calcium deficient apatite precipitation. Additionally, PLS also enhanced the precipiation and remineralization of the demineralized dentine (Gandolfi et al., 2011; Kangwankai et al., 2017). It could be speculated that remineralization of the adjacent uncoated dentine, induced by bioactive materials, would have been less effective through the hybrid layer formed by convential adhesive systems. This speculation will be tested in further study along with micro-leakage experiment.

Renewal MI has been designed to be an alternative material for disadvantaged individuals, in which clinical outcome has always been compromised, for example due to access to oral care by specialists and difficulties in patient management. Benefits of this novel dental composite, as a self-adhesive restorative material, can provide a simpler, less time-consuming, and less technique-sensitive clinical procedure, which sounds convincing for paediatric and special needs patients (Gayatri et al., 2018; Tuloglu, Tunc, Ozer, & Bayrak, 2014) as well as for the areas with limited resources or during the time of pandemic crisis. Before the clinical criteria could be established for the Renewal MI, certain properties must be tested and modified to meet the clinical requirements

6. Conclusion

Monocalcium phosphate addition in dental composite, when aged in artificial saliva, induced the swelling and precipitation at the interface, resulting in the reduction of micro-gap formation at the interface of tooth structure. Therefore, dental composite with MCP could progressively increase sealing ability, without prior bonding agent used.

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